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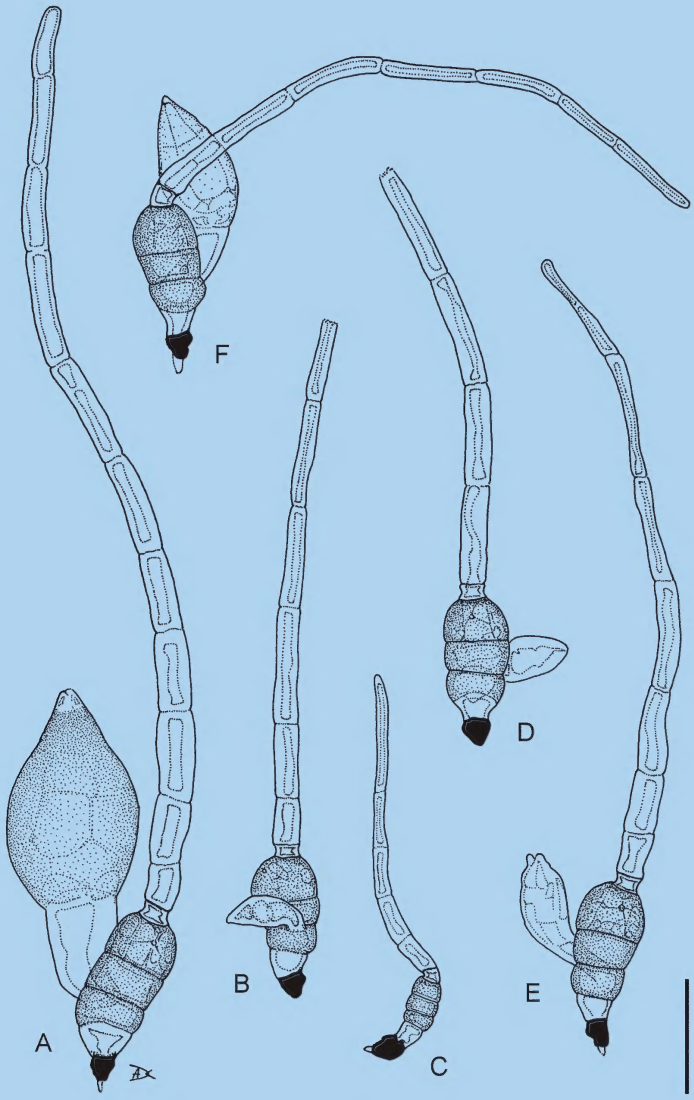

Cantharomyces elongatus sp. nov.
(Haelewaters & De Kesel— PLATE 1, p. 459)
ANDRÉ DE KESEL, *artist*

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Errata from volume 122

p.81, line 4	for: (No. U0836604)	read: (No. U1202262)
p.416, line 4 in Fig.	for: *Cantharellus atratus* (AB445115)	
	read: *Craterellus atratus* (AB445115)	

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From the Editor-in-Chief

Finally! — Despite our valiant efforts to bring Mycotaxon 123 to publication in April as promised, medical science prevented our completing this volume as rapidly as we had hoped. Our apologies to a very patient 203 authors (representing 32 countries!) and 106 expert reviewers – not to mention what we hope are equally patient subscribers.

Within the pages of the delayed 2013 January–March volume are three new genera (*Circinoconiopsis* from Brazil, *Cordochaete* from India, *Yunchangia* from China) and 57 species new to science representing *Acaulospora, Fusticeps*, *Neolinocarpon, Passalora*, and *Pseudocercospora* from Brazil; *Alternaria, Aureoboletus, Chloridium*, *Coccomyces, Gautieria, Hymenoscyphus, Inocybe, Lecanora, Lobothallia, Lyophyllum, Marasmius, Miriquidica,* and *Tuber* from China; *Cantharomyces* from the Netherlands; *Cortinarius, Ellisembia,* and *Phellodon* from the U.S.A.; *Endocarpon* from Korea; *Endophragmiella* and *Sporormiella* from Spain; *Entocybe* from Canada; *Heliocephala* from Vietnam; *Inocephalus* from Australia; *Inonotus* and *Stropharia* from India; *Lichenochora* from Peru; *Lignosus* from Malaysia; and *Pestalotiopsis* and *Scleroderma* from Thailand.

In addition to range extensions and/or new hosts for previously named taxa, we also offer new combinations in *Gymnopus, Hygrocybe, Ophiodothella,* and *Scheffersomyces,* newly established synonymies, and conclusions from recent ultrastructural (*Dacrymycetales*) and molecular (*Collectotrichum*) studies.

Annual plea from your over-worked Editors — There is too little of us to go around! In this age of instant communication, overly enthusiastic writers need to re-read their manuscripts for mistakes, confusion, mangled syntax, and errors. Your editors spend a minimum of four to five hours per manuscript checking nomenclature, science, formatting, and grammar, so it is not unreasonable to ask our contributors to spend more time writing, formatting, and reading each manuscript before submission. Extra lines should not appear between text. Initial tab sets should NEVER be used. All hyperlinks MUST be made inactive (NEVER copy and paste text from the Internet!). But most importantly, please consult a native English-speaker to ensure that the text makes sense. Finally, we ask every co-author to read the text at least ten times before first submission.

This will not only help keep us on schedule, the editors will be much, much happier and tranquil. Who knows – with your help, we might even be able to publish all four volumes before the end of this year!

Warm regards,

Lorelei Norvell (*Editor-in-Chief*)
24 July 2013

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Scleroderma suthepense, a new ectomycorrhizal fungus from Thailand

Jaturong Kumla, Nakarin Suwannarach, Boonsom Bussaban & Saisamorn Lumyong*

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Abstract — A new species of earthball, described herein as *Scleroderma suthepense,* was found under *Prunus cerasoides* in Doi Suthep-Pui National Park, Thailand. Morphological characteristics indicate it belongs to section *Scleroderma*. Molecular analysis showed differences from previously described *Scleroderma* species. A description, illustration, and comparisons with related taxa are provided.

Key words — gasteromycete, phylogenetic analysis, taxonomy

Introduction

Ectomycorrhizal fungi have symbiotic associations with many tree species in forests around the world and over 7000 species have been described (Taylor & Alexander 2005). *Scleroderma* (*Boletales*; Binder & Hibbett 2006) has worldwide distribution in temperate and tropical regions and forms ectomycorrhizas with a broad range of woody plants including members of families *Caesalpiniaceae*, *Dipterocarpaceae*, *Fagaceae*, *Myrtaceae*, *Phyllanthaceae*, and *Pinaceae* (Munyanziza & Kuyper 1995; Sims et al. 1997; Sanon et al. 2009). There are 131 *Scleroderma* names in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp). In Thailand, only ten species of *Scleroderma* have been reported, *S. areolatum*, *S. bovista*, *S. cepa*, *S. citrinum*, *S. dictyosporum*, *S. flavidum*, *S. lycoperdoides*, *S. polyrhizum*, *S. sinnamariense*, and *S. verrucosum* (Chandrasrikul et al. 2011). *Scleroderma* species have been used to increase the growth of tree seedling, both in nurseries and in the field (Chen et al. 2006). Several species of *Scleroderma* have been proposed based on morphological characteristics of their basidiomes and basidiospores (Guzmán 1970; Sims et al. 1995; Guzmán et al. 2004). Recent studies have also used molecular analysis to analyze inter- and intraspecific variation in *Scleroderma* species (Phosri et al. 2009; Nouhra et al. 2012).

During an investigation of ectomycorrhizal fungi associated with tree species in northern Thailand, we found an interesting species of *Scleroderma* under *Prunus cerasoides* Buch.-Ham. ex D. Don (*Rosaceae*), which we describe as a new species in the present paper. To confirm their taxonomic status the phylogenetic relationship among 25 isolates of *Scleroderma* were also determined by analysis of complete sequences of the internal transcribed spacer (ITS) regions of the RNA gene.

Materials & methods

Morphology studies

Basidiomes of *Scleroderma* were collected from Medicinal Plant Garden (18°48′20″N 98°54′52″E, elevation 1072 m), Doi Suthep-Pui National Park, Chiang Mai Province, Thailand in May 2012. Basidiomes were wrapped in aluminum foil or kept in plastic specimen boxes until transport back to the laboratory where notes on macromorphological features and photographs were taken within 24 h. Macromorphological data were derived from fresh specimens, whereas micromorphological data were derived from dried specimens mounted in 95% ethanol followed by distilled water, 3% KOH or Melzer's reagent. Size data of anatomical features are based on at least 50 measurements of each structure. The specimens were dried at 40–45 °C and deposited at the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of a dried specimen was extracted according to a CTAB method (Kumla et al. 2012). The ITS regions of the RNA gene were amplified by polymerase chain reaction (PCR) with primers ITS4 and ITS5 under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min. The PCR products were then checked on 1% agarose gels stained with ethidium bromide and visualized under UV light. PCR products were purified by using PCR clean up Gel extraction NucleoSpin® Extract II Purification Kit (Macherey-Nagel, Germany, Catalog no. 740 609.50) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sequencing reactions were preformed and the sequences were automatically determined in the genetic analyzer (1ST Base, Malaysia) using the PCR primers mentioned above. Sequences were used to query GenBank via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html). For the phylogenetic analysis, a multiple alignment subroutine in Clustal X (Thompson et al. 1997) and a maximum-parsimony analysis using the PAUP beta 10 software version 4.0 (Swofford 2002) were carried out.

Results

Taxonomic description

Scleroderma suthepense Kumla, Suwannarach & Lumyong, **sp. nov.** PLATE 1

MycoBank MB800878

Differs from *Scleroderma meridionale* by its longer basidiospore spine length.

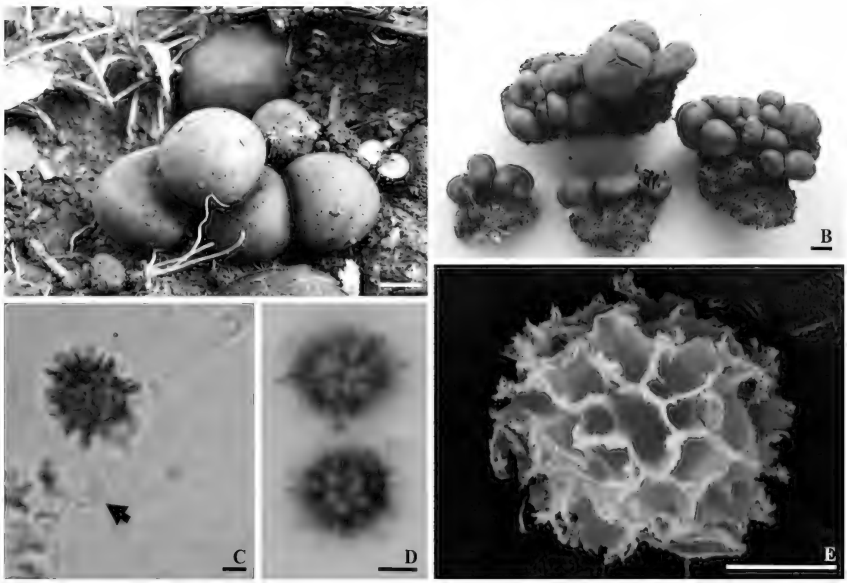

Plate 1. *Scleroderma suthepense*. A, B: Basidiomes. C: Hypha with a clamp connection (arrowed). D: Basidiospores as observed under a compound microscope. E: Basidiospore as observed with a scanning electron microscope. Scale bars: A, B = 1 cm; C–E = 5 μm.

Type—Thailand, Chiang Mai Province, Muang District, Doi Suthep-Pui National Park, Medicinal Plant Garden, 18°48′20″N 98°54′52″E, elevation 1072 m, rainforest dominated by *Prunus cerasoides*, *Cinchona pubescens*, and other trees, on sandy loam, 15 May 2012, Jaturong Kumla & Nakarin Suwannarach (**Holotype**, SDBR-CMU55-SC2; GenBank sequence, JX205215).

Etymology—*suthepense*, referring to Doi Suthep-Pui National Park, where the new species was found.

Basidiomes globose or subglobose 1.1–3.5 cm diameter, 1.0–3.9 cm height, rhizomorphs well developed, white to yellow, 0.5–1.2 cm height. Peridium 0.5–1.0 mm thick when fresh, leathery, surface partially smooth with scattered, small and thin scales, greyish yellow to greyish brown, consisting of two layers. The outer layer consists of cylindrical, thick-walled, yellowish brown hypha up to 8.0 μm diameter, with scattered clamp connections, turning to reddish brown with KOH. The inner layer consists of cylindrical, thick-walled, hyaline hyphae up to 6.0 μm diameter, with clamp connections. Gleba when mature dark greyish brown to back and pulverulent. Basidia not observed. Basidiospores globose to subglobose, strongly reticulate with spines, 8.0–13.0 μm diameter (n = 50) including ornamentation (Plate 1D,E), spine 1.0–2.5 μm in length, dark yellowish brown in water or KOH and not changing in Melzer's reagent.

Ecology & distribution — Terrestrial on sandy loam, under *Prunus cerasoides* in dipterocarp forest; known only from Thailand.

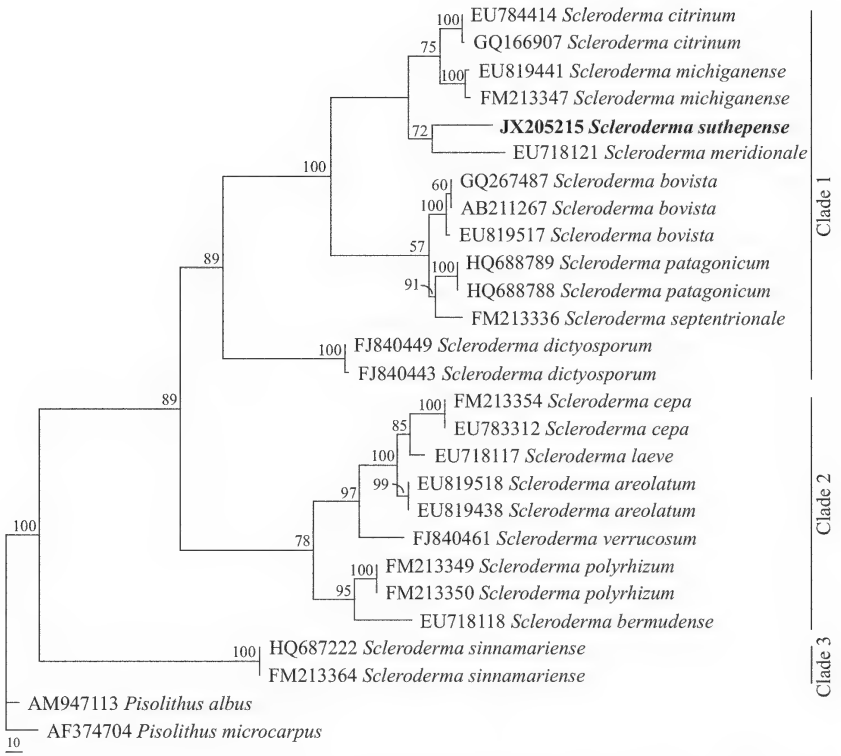

Plate 2. A maximum parsimonious tree inferred from a heuristic search of ITS 1, 5.8S ribosomal RNA gene, and ITS 2 alignments of 27 sequences. *Pisolithus albus* and *P. microcarpus* were used to root the tree. Branches with bootstrap values ≥50% are shown above each branch and the bar represents 10 substitutions per nucleotide position.

Molecular analysis

The 679-bp ITS sequence of *S. suthepense* (JX205215) was deposited in GenBank and analyzed phylogenetically with 24 other *Scleroderma* sequences and the outgroup (*Pisolithus albus* and *P. microcarpus*) obtained from the GenBank database. Heuristic searches produced a tree length of 803 steps, CI = 0.685, RI = 0.823, RC = 0.563 and HI = 0.315. One maximum-parsimony tree is shown in Plate 2. A phylogenetic dendrogram separates *Scleroderma* fungi into three clades. Clade 1 contains species with reticulate spores (sect. *Scleroderma*), while clades 2 and 3 include species with spiny or subreticulate spores. *Scleroderma suthepense* stands within clade 1 together with *S. bovista*,

S. citrinum, *S. dictyosporum*, *S. meridionale*, *S. michiganense*, *S. patagonicum*, and *S. septentrionale* and forms a sister taxon with *S. meridionale* (72% bootstrap support). Clade 2 includes six species (*S. areolatum*, *S. bermudense*, *S. cepa*, *S. laeve*, *S. polyrhizum*, *S. verrucosum*). Clade 3 comprises a single species, *S. sinnamariense*.

Discussion

Based on basidiospore morphology, *Scleroderma* species are separated into three sections; sect. *Aculeatispora* (spiny spore), sect. *Sclerangium* (subreticulate spore), and sect. *Scleroderma* (reticulate spore) (Guzmán 1970; Sims et al. 1995, 1997). However, it is difficult to distinguish *Scleroderma* species based only on morphology because basidiome size and shape change depending on the soil and environment (Sanon et al. 2009). *Scleroderma suthepense*, described as a new species in the present study, was found beneath *Prunus cerasoides* and is placed in sect. *Scleroderma* based on its reticulate basidiospores and hyphal clamp connections. Its basidiospores are similar in size to those of *S. dictyosporum*, *S. meridionale,* and *S. septentrionale* (Table 1). Our ITS sequence analysis clearly separates *S. suthepense* from the other reticulate spored *Scleroderma* species in sect. *Scleroderma,* although *S. suthepense* appears closely related to *S. meridionale*, which has a shorter basidiospore spine length (0.5–1.5 µm) and is reported only in North America (Sims et al. 1995; Guzmán & Ovrebo 2000). Our analysis separates the reticulate spored clade (clade 1) and the spiny or subreticulate spored clades (clades 2 and 3) with 89% bootstrap support (Plate 2). This result was similar to previous molecular phylogenetic studies, which separated *Scleroderma* species into two moderately to strongly supported clades (Phosri et al. 2009; Nouhra et al. 2012). Further studies are required to confirm an ectomycorrhizal synthesis between *S. suthepense* and various host plants.

Table 1. Basidiospore sizes of *Scleroderma suthepense* and other species in *Scleroderma* sect. *Scleroderma*.

Species	Spore diameter (µm)	References
S. bovista	11–16	Sims et al. (1995), Cortez et al. (2011)
S. citrinum	11–16	Sims et al. (1995), Cortez et al. (2011)
S. dictyosporum	9–14	Guzmán (1970), Cortez et al. (2011)
S. hypogaeum	17–20	Sims et al. (1995)
S. meridionale	8–17	Sims et al. (1995), Guzmán & Ovrebo (2000)
S. michiganense	14–20	Sims et al. (1995)
S. minutisporum	4–7	Alfredo et al. (2012)
S. patagonicum	19–28	Nouhra et al. (2012)
S. septentrionale	8–15	Jeppson & Piatek (2005)
S. suthepense	8–13	Present study

Such knowledge is important for selection and management of ectomycorrhizal fungi for greenhouse and in field inoculation programs in Thailand.

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Lichenochora tertia (*Phyllachorales*): the third species of the genus growing on *Xanthoria elegans*

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Abstract — *Lichenochora tertia* is a new lichenicolous fungus described from Peruvian Andes. The species is characterized by small perithecia deeply immersed in the thallus of *Xanthoria elegans* and small, narrowly ellipsoidal ascospores with subtle torus along the septum; the fungus does not induce gall formation.

Key words — Neotropics, South America, biodiversity, taxonomy

Introduction

During the study of the lichenicolous biota of Bolivia (Flakus & Kukwa 2012a,b, Kukwa et al. 2012) we examined an infected specimen of *Xanthoria elegans* collected by the fourth author in Peru, which was found to host an undescribed *Lichenochora*. The lichenicolous biota of Peru is still very poorly studied, and the only paper fully dedicated to this subject was published by Etayo (2010). Therefore, it is not surprising that a new taxon was discovered in a biologically rich Andean ecosystem.

The lichenicolous genus *Lichenochora* Hafellner (*Phyllachorales*) comprises 34 species worldwide, of which ten parasitize members of the family *Teloschistaceae*. Two of them are known to grow on *Xanthoria elegans* (Link) Th. Fr. (Triebel et al. 1991, Navarro-Rosinés & Etayo 2001, Etayo & Navarro-Rosinés 2008, Hafellner et al. 2008, Lawrey & Diederich 2011).

Material & methods

The morphology and the anatomy were examined by using a NIKON SMZ800 and a NIKON ECLIPSE 80i (DIC) microscopes. The anatomy was studied in water, KOH solution (K) and, for the ascus structure, in Lugol's iodine solution without (I) or with pre-treatment with KOH (K/I). The measurements were made in tap water. Ascospores measurements are presented as: arithmetic mean – standard deviation, arithmetic mean, and arithmetic mean + standard deviation, flanked by the minimal and maximal measurements in parentheses, and the length/breadth ratio (l/b) is presented in the same way, followed by the number of measurements (n). Values in italics (e.g., *–3.2–*) are arithmetic means. The holotype is conserved in Herbario Nacional de Bolivia, Universidad Mayor de San Andrés, La Paz (LPB).

Results

Lichenochora tertia Etayo, Flakus & Rodr. Flakus, **sp. nov.** PLATE 1

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Differs from *Lichenochora sinapispermae* by its smaller perithecia, deeply immersed in the host thallus and not forming galls, and by its more narrowly ellipsoidal ascospores.

Type — Peru: Dep. Cusco, Prov. Canchis, Cordillera de Vilcanota, cerca del Nevado Japu Punta, vegetación altoandina con suelo muy pedregoso con rocas de gran tamaño y poca cobertura de plantas superiores, 5320 m, 13°45′41.4″S 71°04′58.8″W, en *Xanthoria elegans* en piedra silícea, 13 March 2008, P. Rodriguez Flakus 498b (**Holotype**, LPB).

Etymology — *tertius* (Lat.), referring to this being the third species of the genus *Lichenochora* found on *Xanthoria elegans*.

Fungus parasymbiotic, not inducing gall formation. Mycelium hyaline, inconspicuous, observed only at the base of the perithecia. Ascomata perithecia dispersed or aggregated in small groups, 120–190 µm in diam. and up to 200 µm high, pyriform, strongly rough near apical part, immersed in 2/3 with only the upper part erumpent outside of host thallus; forming papillae. Exciple dark reddish-brown (K+ olive-brown), 20–40 µm wide, formed by several layers of polygonal cells, usually with many oil guttules in internal cells, of dark brown thick-walled cells, 8–15 µm in diam., with some cells protruding to the outside giving it a verrucose appearance; innermost excipular layer of pale brown to hyaline thin walled cells, 6–15 × 2–10 µm. Papillae of elongate cells formed mainly in the upper part of perithecium (near ostiole), usually grouped and forming wide appendages up to 20–35 um long. Hymenium inspersed by oil droplets, 1–8 µm in diam., large droplets (5–8 µm in diam.) abundant and obscuring the hymenium. Paraphyses rare in mature ascomata and obscured by oil droplets, 3–8 µm wide, with thin-walled cells. Asci (2–)6–8-spored, 60–90 × 11–15 µm, clavate, functionally unitunicate, K/I–, with stalk up to 5 µm long, thin-walled, not thickened in the apex. Ascospores narrowly ellipsoidal, sometimes slightly curved, with obtuse to slightly tapering ends, smooth-walled, (0–)1-septate, colourless, without perispore, formed

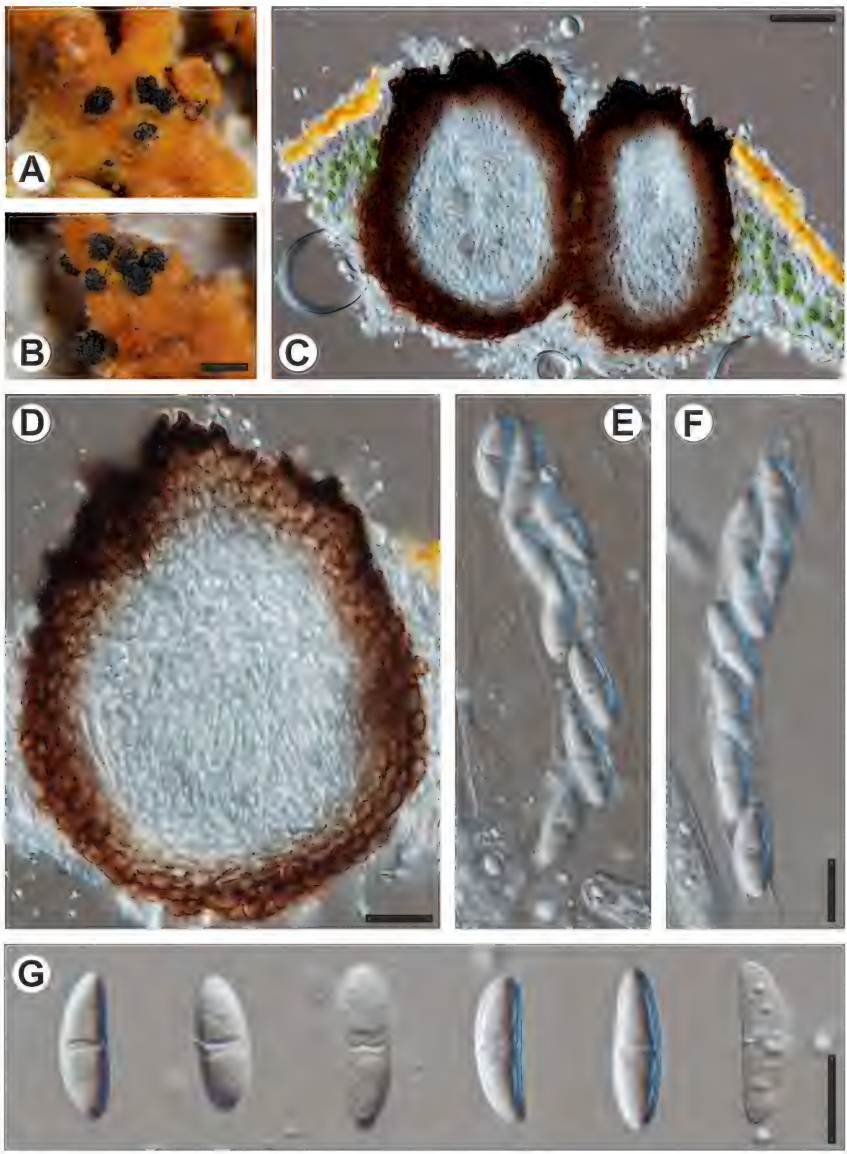

Plate 1. *Lichenochora tertia* (holotype). A, B: habit of perithecia with rugose surface of *Xanthoria elegans*; C: section through two perithecia immersed in host thallus and forming wide erumpent appendages in upper part; D: section of perithecium showing structure of excipulum; E, F: asci with ascospores inside; G: ascospores. Scale bars: A, B = 200 µm; C = 50 µm; D = 25 µm; E–G = 10 µm.

subtle torus along the septum, and usually with one large oil guttule in each cell (sometimes splitting up into several small ones), walls c. 0.5 µm thick, (12–)15–*16.5*–18(–20) × (4–)4.9–*5.3*–5.7(–6.5) µm, l/b = (2.2–)2.8–*3.2*–3.6 (–4.5) (n = 120).

Distribution and habitat — The new species is known only from the type locality in Peruvian Andes, where it was found on epilithic *Xanthoria elegans* in high montane conditions.

Comments — *Lichenochora tertia* is characterized by its small perithecia, small, narrowly ellipsoidal ascospores with a subtle torus along the septa, and not gall-forming. The most similar species is *L. sinapispermae* Etayo & Nav.-Ros. due to its ascospore size and the presence of the torus, but it has comparatively wider ascospores, (5.5–)6–*6.9*–8(–8.5) µm, which are broadly ellipsoidal, with l/b ratio (1.6–)1.9–*2.2*–2.5(–2.8), comparatively bigger perithecia, 200–250 µm in diam. (230–300 µm high), and also shorter papillae, 8–13 µm long (Navarro-Rosinés & Etayo 2001). Moreover, the perithecia are at first half-immersed, but soon almost sessile on the thallus of *Caloplaca sinapisperma* (Lam.) Maheu & A. Gillet.

According to Hafellner et al. (2008) and Etayo & Navarro-Rosinés (2008) two *Lichenochora* species are known to inhabit *Xanthoria elegans*, *L. elegantis* Hafellner and *L. xanthoriae* Triebel & Rambold. *Lichenochora elegantis* has longer ascospores, 28–33 µm long, 4-spored asci and much bigger perithecia, 300–400 µm in diam. (Hafellner et al. 2008), whereas in *L. xanthoriae* ascospores are considerably wider, 8–9(–9.5) µm wide, and the species induces the formation of small cecidia (Triebel et al. 1991).

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Inonotus tramisetifer (*Agaricomycetes*), a new species from India

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Abstract – A new species, *Inonotus tramisetifer (Hymenochaetaceae)*, is described in association with *Quercus incana* from the state of Himachal Pradesh in India.

Key words – Narkanda, tramal setal hyphae

While conducting fungal forays in Narkanda area of district Shimla, Himachal Pradesh (India), Harpreet Kaur and G.S. Dhingra collected a poroid fungus associated with tree of *Quercus incana*. After comparison of macroscopic and microscopic features (Sharma 1995, Ryvarden 2004, 2005) it has been identified as a new species of *Inonotus* close to *I. cuticularis* (Bull.) P. Karst., *I. farlowii* (Lloyd) Gilb., and *I. munzii* (Lloyd) Gilb., having branched pilear setal hyphae along with monomitic hyphal system and yellowish brown basidiospores.

Inonotus tramisetifer Har. Kaur, Avneet P. Singh & Dhingra, **sp. nov.** Figs 1–10

MycoBank 800985

Differs from *Inonotus cuticularis, I. farlowii,* and *I. munzii* by its tramal setal hyphae.

Type: India, Himachal Pradesh: Narkanda, on way to Rampur, on living tree of *Quercus incana* Bartram (*Fagaceae*), 13 August 2011, Harpreet 4746 (PUN, **holotype**).

Etymology: The epithet refers to the presence of tramal setae.

Basidiocarps annual, pileate, poroid, effused-reflexed to dimidiate to applanate, solitary to imbricate, up to 4 cm wide, 3 cm in radius, and 1.5 cm thick near base, with brownish orange exuding droplets; abhymenial surface smooth to velutinate, azonate, yellowish white to light orange to brown when fresh becoming glabrous, light orange to bark brown on drying; hymenial surface poroid, grayish yellow when fresh becoming dark blonde to clay brown on drying; pores round to angular, 3–5 per mm; margins obtuse, yellowish white when fresh becoming dark brown on drying, sterile ≤1 cm; pore tubes ≤1 mm

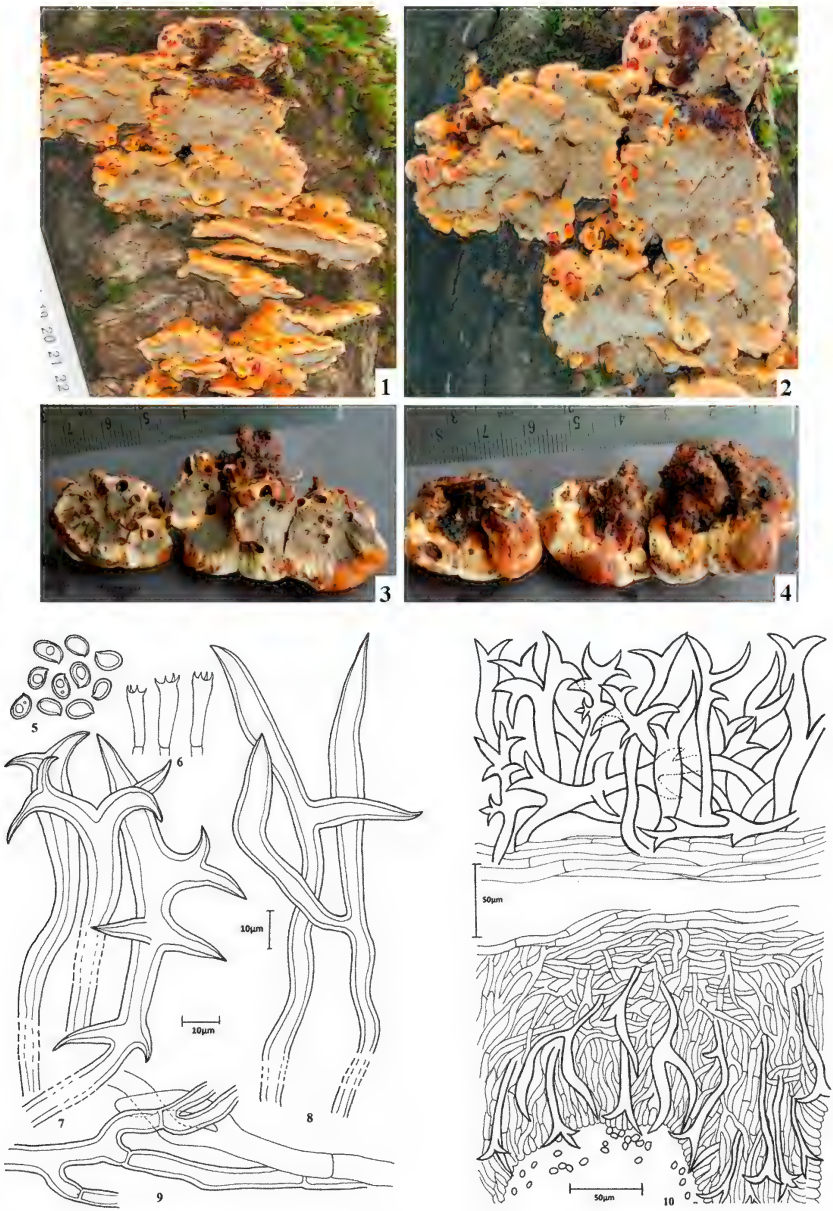

Plate 1. *Inonotus tramisetifer* (holotype). Figs 1–4: Basidiocarps. 1. Association with host. 2. Basidiocarp exuding droplets. 3. Hymenial surface. 4. Abhymenial surface. Figs 5–10: Microscopic structures. 5. Basidiospores. 6. Basidia. 7. Pilear setal hyphae. 8. Tramal setal hyphae. 9. Generative hyphae. 10. Section.

long, entire, brownish orange; dissepiments thin to thick, entire; context ≤1 cm thick, firm-fibrous, brownish orange, upper tomentum delimited by a darker compact layer that is formed by setigerous elements. Hyphal system monomitic; generative hyphae ≤7.1 µm wide, branched, septate, without clamps, thin- to thick-walled, pale yellowish to yellowish brown. Setal hyphae of two types 1) pilear setal hyphae ≤1350 × 11 µm, abundant, monopodial to dichotomously branched, branches with curved tips, thick-walled, dark brown; 2) tramal setal hyphae ≤1300 × 6 µm, abundant, monopodial to dichotomously branched, branches with curved tips, extending into the hymenium ≤19 µm and giving the misleading appearance of hooked hymenial setae, thick-walled, brown to dark brown. Hymenial setae absent. Basidia 13–16.5 × 4–4.7 µm, clavate, 4-sterigmate, without basal clamp; sterigmata up to 2.4 µm long. Basidiospores 5.9–6.5 × 3.5–4.7 µm, broadly ellipsoidal, thick-walled, with oil droplets, yellowish brown, inamyloid, acyanophilous.

Remarks— *Inonotus tramisetifer* is similar to *I. cuticularis*, *I. farlowii*, and *I. munzii,* but *I. cuticularis* and *I. farlowii* differ by their hymenial setae and lack of tramal setal hyphae, and *I. munzii* differs by its duplex context and lack of tramal setal hyphae (Ryvarden 2004, 2005).

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Three new species of *Hymenoscyphus* from tropical China

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Abstract — Three new species of *Hymenoscyphus* collected from tropical regions of China are described and illustrated. *Hymenoscyphus magnicellulosus* possesses whitish sessile apothecia with large-celled textura prismatica at flank and isodiametric cells at the base, and inequilateral ellipsoid ascospores 12.5–19(–22) × 4.5–6.7 µm. *Hymenoscyphus uniseptatus* has white stipitate apothecia, rectangular ectal excipular cells, broadly clavate asci, and broadly fusoid 1-septate ascospores 16.7–20.5 × 5–6.5 µm. *Hymenoscyphus yunnanicus* is characterized by cream to light yellow stipitate apothecia, narrow ectal excipular hyphae, and multiguttulate fusoid ascospores 16.5–19.5 × 5–5.6 µm. Comparisons were made among the new species and their morphologically similar fungi.

Key words —*Helotiales*, morphology, taxonomy

Introduction

Hymenoscyphus Gray (*Helotiaceae*) is characterized by discoid and stipitate to sessile apothecia, a white to yellowish hymenium surface, an ectal excipulum of textura prismatica or sometimes mixed with textura angularis, a medullary excipulum of textura intricata to textura porrecta, ascospores that are fusoid, ellipsoid to scutuloid, and growth on plant materials (Gray 1821; Dennis 1956; Sharma 1991; Lizoň 1992; Zhang 2002). The main characters used to distinguish species are color, shape and size of apothecia, structure of ectal excipulum, size and apical apparatus of asci, shape, size and guttulation of ascospores, and sometimes substrate specificity (Phillips 1887; Verkley 1993; White 1943; Dennis 1956; Dumont 1981a,b; Lizoň 1992).

Regional studies of *Hymenoscyphus* were carried out in Europe (Phillips 1887; Velenovský 1934; Dennis 1956, 1962, 1968; Lizoň 1992; Milekhin & Prokhorov 2008), America (Seaver 1951; Dennis 1954, 1958a, 1960; Dumont & Carpenter 1982; Gamundi 2003), Asia (Thind & Singh 1970, 1972; Thind & Sharma 1980; Sharma 1991; Zhang 2002; Han & Shin 2008), Oceania

(Dennis 1958b, 1961), and Africa (Dennis 1984; Descals et al. 1984; Fisher & Spooner 1987). More than 500 names have been connected to *Hymenoscyphus* (http://www.indexfungorum.org/Names/Names.asp), and approximately 155 species are currently accepted (Kirk et al. 2008). The genus needs a monographic treatment.

Twenty-six species are known from China (Teng 1934, 1963; Tai 1979; Zang 1983; Korf & Zhuang 1985; Zhuang & Korf 1989; Bi et al. 1990; Zhuang 1995, 1998a,b, 1999a,b, 2001, 2003a,b; Zhuang & Wang 1998a,b; Yu et al. 2000; Wang & Pei 2001; Zhang 2002; Zhang & Zhuang 2002a,b, 2004). The species previously described from China were mainly based on materials from temperate areas (Zhang 2002; Zhang & Zhuang 2002a,b, 2004), and very few species were known from tropical China.

During our examinations of the *Hymenoscyphus* collections from tropical Hainan and Yunnan provinces, we reported six species (Zheng & Zhuang 2011), of which five occur also in temperate China. As a continuation of the work, we describe and name three new species in this paper: *H. magnicellulosus*, *H. uniseptatus* and *H. yunnanicus*. Distinctions among the new species and their related fungi are discussed.

Materials & methods

Specimens examined were collected from tropical areas of Hainan and Yunnan provinces in 1999 and 2000. Field notes were taken from apothecia in fresh condition. Dried apothecia were rehydrated with distilled water and sectioned at a thickness of 10–20 μm with a freezing microtome (YD-1508A, Yidi Medical Instrument Co., Jinhua, China). Measurements were taken from longitudinal sections and from squash mounts in lacto-phenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Iodine reactions of ascus apparatus were tested in Melzer's reagent with 3% KOH aqueous solution pretreatments. Features of apical ring were recorded according to Baral (1987). Ascospore guttulation was examined in 3% KOH aqueous solution. Photographs were taken using a Canon G5 digital camera (Tokyo, Japan) connected to a Zeiss Axioskop 2 Plus microscope (Göttingen, Germany) and a Zeiss Stemi 2000C (Göttingen, Germany) stereomicroscope. Line drawings were made using an Opton microscope (West Germany) equipped with a drawing tube. Specimens examined are deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Taxonomy

Hymenoscyphus magnicellulosus H.D. Zheng & W.Y. Zhuang, **sp. nov.**

MycoBank MB564248 Figs 1A, 2, 5A–C

Differs from *Hymenoscyphus tamaricis* by its much smaller whitish sessile apothecia, narrower medullary hyphae, and shorter and wider asci.

Type: China, Yunnan: Pingbian, Daweishan, alt. 1900 m, on rotten bark, 4 Nov 1999, W.Y. Zhuang & Z.H. Yu 3254 (**Holotype**, HMAS 188555).

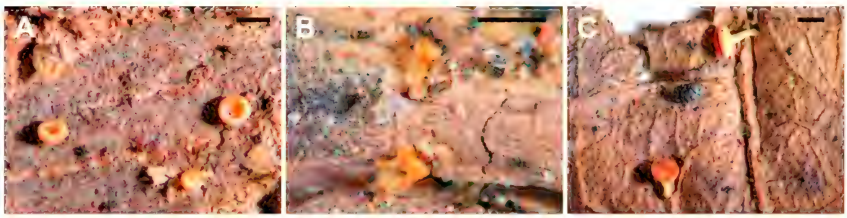

FIGURE 1 Gross morphology of dried apothecia on natural substrates. A. *Hymenoscyphus magnicellulosus* (holotype); B. *H. uniseptatus* (holotype); C. *H. yunnanicus* (holotype). Scale bars = 0.5 mm.

ETYMOLOGY: The specific epithet refers to the large cells in ectal excipulum.

Apothecia turbinate, subsessile to sessile, scattered or 2–5 in a cluster, margin smooth, 0.6–2 mm in diam.; hymenium surface flat to convex, drying slightly to deeply concave, whitish when fresh, drying pale tan; receptacle surface smooth, paler than hymenium. Outer covering layer absent. Ectal excipulum 20–55 μm thick, of textura porrecta at margin, hyphae 3–5 μm wide; becoming textura prismatica at flank, cells 8–30 × 4–11 μm; and of textura angularis towards the base, cells up to 25 × 11 μm, or 5–15 μm in diam. if isodiametric, cells hyaline, thin- to slightly thick-walled. Medullary excipulum of textura intricata, 30–250 μm thick, hyphae hyaline, 1.5–4 μm wide, thin-walled. Subhymenium not distinguishable. Hymenium 95–150 μm thick. Asci 8-spored, 88–129 × 9–12 μm, cylindric-clavate, gradually narrowed into a short-medium stalk, base attenuate, arising from croziers, apex round to broadly papilliform, apical thickening 2–3 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring weakly to moderately bluing as two lines about 1–1.5 μm long, even in width or slightly thinner upward, of the *Hymenoscyphus*-type. Ascospores 12.5–19(–22) × 4.5–6.7 μm, obliquely uniseriate to irregularly biseriate, hyaline, ellipsoid, slightly flattened on one side, occasionally 1-septate, with a dark stained area in cotton blue, containing 1–2 large oil drops and several small ones in cotton blue and KOH solution; guttules ellipsoid, rounded to irregular in shape, individuals up to 10 × 5 μm. Paraphyses filiform, slightly swelling and 2–3 μm at the apex, 1–2 μm at lower part, equal to or slightly exceeding the asci.

ADDITIONAL SPECIMENS EXAMINED: China, Hainan: Changjiang, Bawangling, alt. 1150 m, on rotten wood of broad-leaved tree, 7 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3669 (HMAS 188556); Lingshui, Diaoluoshan, alt. 1080 m, on rotten wood of broad-leaved tree, 14 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3866 (HMAS 188557); Tongza, Wuzhishan, alt. 1600 m, on rotten wood of broad-leaved tree, 16 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3876 (HMAS 188558).

NOTES: All examined *H. magnicellulosus* specimens share very similar macroscopic and microscopic characteristics.

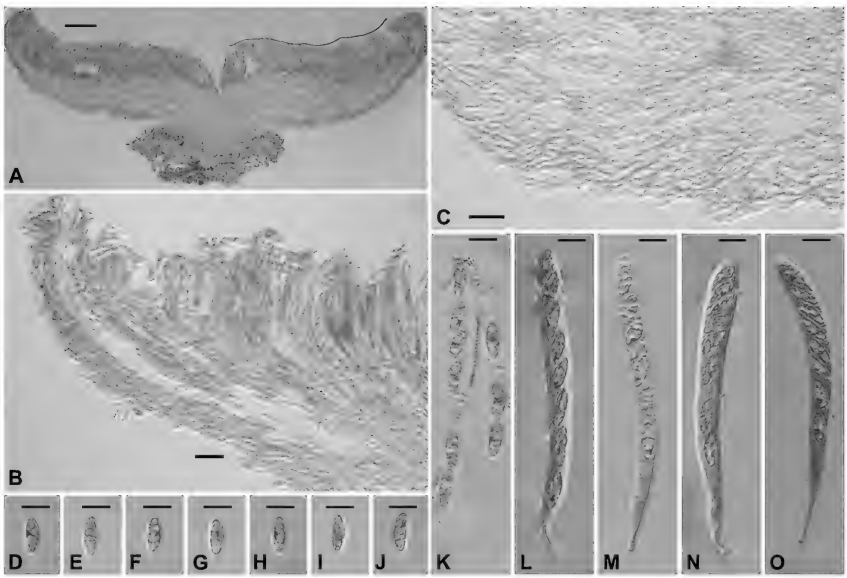

FIGURE 2 *Hymenoscyphus magnicellulosus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium at margin; C. Excipular structure at flank and near base; D–J. Ascospore (E. 1-septate ascospore); K. Ascospores in asci; L–O. Asci. Scale bars: A = 100 μm, B, C = 20 μm, D–O = 10 μm.

Hymenoscyphus tamaricis R. Galán, Baral & A. Ortega resembles *H. magnicellulosus* in lignicolous habit, ectal excipulum structure, asci arising from croziers, and guttulate and occasionally 1-septate ascospores of similar size and shape, but differs in stipitate, pale to light yellowish-ochraceous apothecia which are much larger [(1–)1.5–7(–9) mm in diam.], broader medullary hyphae (3–9 μm wide), longer and narrower asci (125–140 × 8.2–8.5 μm), and restricted only on *Tamarix* host (Galán & Baral 1997).

A species with similarly sized white apothecia and isodiametric cells in ectal excipulum is *H. immutabilis* (Fuckel) Dennis, which can be distinguished from *H. magnicellulosus* by short-stipitate apothecia, smaller and fusoid ascospores (10–13 × 4–4.5 μm) biseriate in smaller asci (80–100 × 8–9 μm), and mainly foliicolous habit (White 1943; Dennis 1956; Dumont 1981b).

Hymenoscyphus fagineus (Pers.) Dennis has whitish and sessile to subsessile apothecia similar to those of *H. magnicellulosus*. It is distinct in ectal excipulum composed of short rectangular cells lying at a high angle to the surface, smaller asci (65–80 × 8–9 μm) J+ and with pore strongly blue in Melzer's reagent, smaller ascospores (8–16 × 4–5 μm), and occurring only on fallen *Fagus sylvatica* pericarps (Dennis 1956; Lizoň 1992).

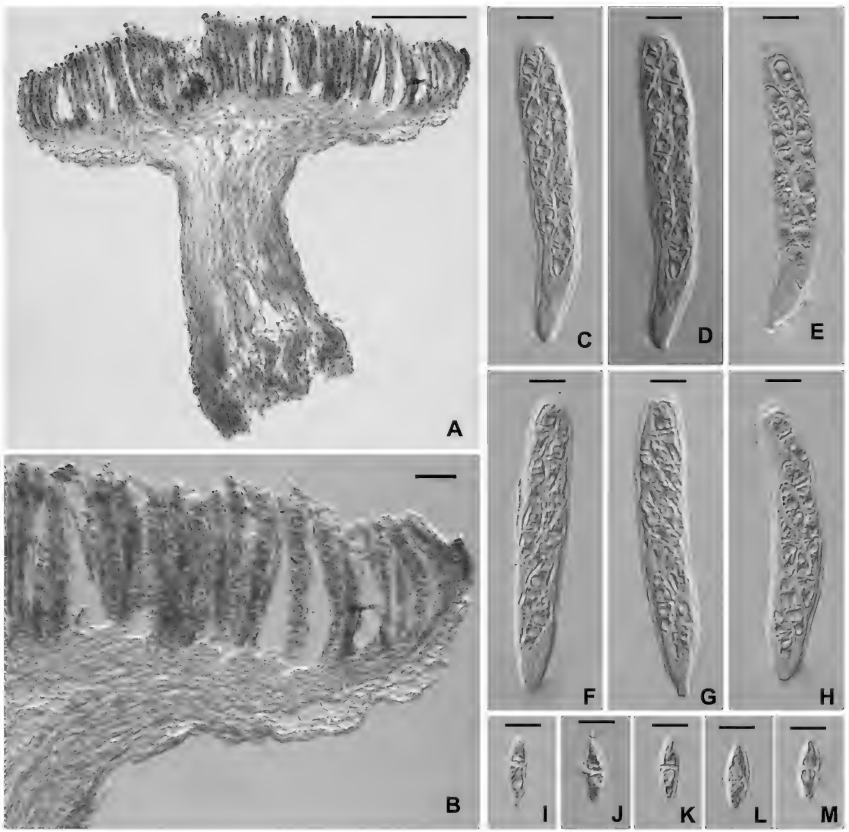

FIGURE 3 *Hymenoscyphus uniseptatus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium; C–H. Asci; I–M. Ascospore. Scale bars: A = 100 μm, B = 20 μm, C–M = 10 μm.

Hymenoscyphus uniseptatus H.D. Zheng & W.Y. Zhuang, **sp. nov.**

MycoBank MB564250 FIGS 1B, 3, 5D–F

Differs from *Hymenoscyphus jinggangensis* by its smaller apothecia, larger asci, and larger 1-septate multiguttulate ascospores.

TYPE: China, Yunnan: Malipo, Nanwenhe, alt. 900 m, on herbaceous stems and rotten wood of broad-leaved tree, 9 Nov 1999, W.Y. Zhuang & Z.H. Yu 3356 (**Holotype**, HMAS 188559).

ETYMOLOGY: The specific epithet refers to the uniseptate ascospores.

Apothecia stipitate, scattered or 2–3 in clusters, round, margin smooth, 0.7–1 mm in diam., stipe 0.3–0.8 mm long; hymenium surface flat to discoid, white when fresh, becoming dull orange when dry; receptacle surface smooth, concolorous with hymenium. Outer covering layer absent. Ectal excipulum of textura prismatica, 15–30 μm thick, cells hyaline, slightly thick-walled,

rectangular to short rectangular, 10–22 × 7–15 μm. Medullary excipulum with two layers, outer layer of textura porrecta, 5–20 μm thick, inner layer of textura intricata, 15–30 μm thick, hyphae hyaline, 2–3 μm wide. Subhymenium not distinguishable. Hymenium 90–105 μm thick. Asci 8-spored, 80–95 × 8.5–14 μm, broadly clavate, base attenuate, arising from simple septa, apex round to conical, apical thickening 3–6 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring weakly bluing as two lines about 1.5–3 μm long, equal to or becoming thinner upward, of the *Hymenoscyphus*-type. Ascospores 16.7–20.5 × 5–6.5 μm, biseriate to irregularly uniseriate, subfusoid, hyaline, 1-septate, containing 3–6 large oil drops and many small ones in cotton blue and KOH solution; guttules round or irregular in shape, individual ones up to 5 × 4 μm. Paraphyses filiform, equal to or slightly exceeding the asci, 1.5–2 μm wide.

Notes: The type specimen of *H. uniseptatus* contains apothecia growing on both herbaceous stems and rotten wood. The macroscopic and microscopic characters of apothecia on different substrates are identical. In view of this, we do not regard *H. uniseptatus* as host-specific.

Hymenoscyphus jinggangensis Yan H. Zhang & W.Y. Zhuang, originally described from temperate China, is most similar to *H. uniseptatus* in stipitate white apothecia, size of ectal excipulum cells, septate ascospores, and lignicolous habit. However, *H. jinggangensis* differs in larger apothecia (1–2.8 mm in diam.), thin-walled ectal excipulum cells, thicker medullary excipulum (up to 235 μm thick), smaller asci (65–83 × 7–8 μm), and smaller (9–17 × 3–5 μm) eguttulate ascospores that are 1–3(–4)-septate instead of uniseptate (Zhang 2002; Zhang & Zhuang 2002a).

Among the known *Hymenoscyphus* species, *H. musicola* (Dennis) Dennis resembles *H. uniseptatus* in stipitate, whitish apothecia, ectal excipulum of large-celled textura prismatica, 1-septate ascospores of similar size, shape and guttulation. It can be easily separated from *H. uniseptatus* by convex apothecial surface, longer asci [(90–)100–110 × 9–12 μm] arising from croziers, and occurrence on *Musa sapientum* (Dennis 1958a; Dumont 1981a).

Hymenoscyphus varicosporoides Tubaki is also similar in stipitate, white apothecia, and 1-septate ascospores, but differs in narrower ectal excipulum hyphae (Tubaki 1966: Pl. II fig. 2), much narrower asci (70–85 × 7–9 μm), smaller ascospores [(10–)12–15(–19) × 4–5(–7) μm] with round ends, and growth on rotting twigs in a semi-aquatic environment (Tubaki 1966).

Hymenoscyphus yunnanicus H.D. Zheng & W.Y. Zhuang, **sp. nov.** Figs 1C, 4, 5G–I
MycoBank MB564251

Differs from *Hymenoscyphus crataegi* by its larger apothecia, smaller ectal excipular cells, larger asci, and larger ascospores.

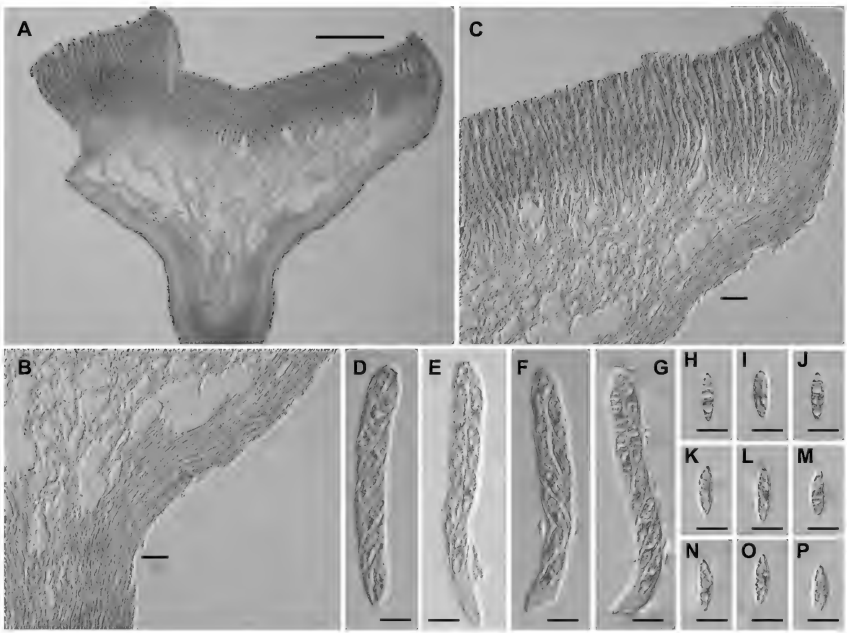

FIGURE 4 *Hymenoscyphus yunnanicus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium; C. Excipular structure between flank and stipe; D–G. Asci; H–P. Ascospore. Scale bars: A = 100 μm, B–C = 20 μm, D–P = 10 μm.

TYPE: China, Yunnan: Xichou, Xiaoqiaogou, alt. 1400 m, on rotten leaves of deciduous tree, 11 Nov 1999, W.Y. Zhuang & Z.H. Yu 3424 (**Holotype**, HMAS 188560); W.Y. Zhuang & Z.H. Yu 3430 (**Isotype**, HMAS 188561).

ETYMOLOGY: The specific epithet refers to the type locality of the fungus.

Apothecia stipitate, scattered, flat to discoid, 0.6–1.5 mm in diam., stipe 0.5–0.8 mm long; hymenium surface cream to light yellow when fresh, drying brownish orange; receptacle surface smooth, paler than hymenium. Outer covering layer of 1–2 cell layers, hyphae 1–2 μm wide. Ectal excipulum of textura prismatica, 15–30 μm thick, cells hyaline, 6–12 × 3–5 μm. Medullary excipulum with two layers, outer layer of textura porrecta, 6–40 μm thick, inner layer of textura intricata, 40–110 μm thick, hyphae hyaline, 2–3 μm wide. Subhymenium not distinguishable (holotype) or 15–30 μm thick (isotype). Hymenium 95–110 μm thick. Asci (75–)87–120 × 7–11 μm, clavate, 8-spored, some with a very short stalk, base attenuate, arising from simple septa, apex round to somewhat truncate, apical thickening 1–2 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring strongly bluing as two lines about 1–1.5 μm long and slightly thicker upward. Ascospores 16.5–19.5 × 5–5.6 μm, obliquely uniseriate to irregularly biseriate, hyaline, fusoid or rounded at anterior end,

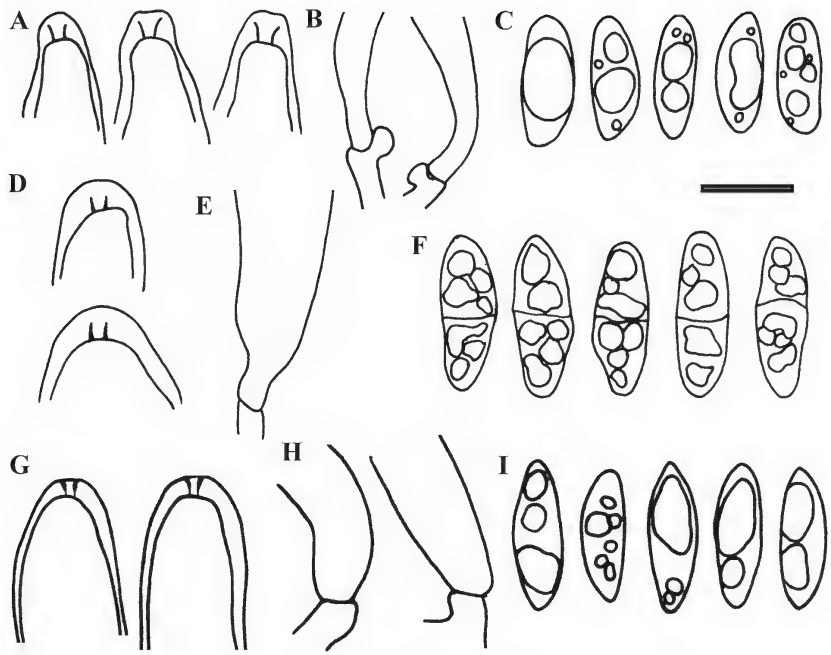

FIGURE 5 Ascospores and asci of *Hymenoscyphus* spp. (holotypes). A–C. *H. magnicellulosus*; D–F. *H. uniseptatus*; G–I. *H. yunnanicus*. A, D, G. Apices of asci in Melzer's reagent; B, E, H. Base of the asci showing croziers or simple septa; C, F, I. Ascospores in KOH. Scale bars = 10 μm.

slightly inequilateral, with a dark stained area in cotton blue, containing 1–3 large oil drops or multiguttulate in cotton blue and KOH solution; guttules rounded or irregular in shape, large ones 2–4 μm in diam. or in width. Paraphyses filiform, equal to or slightly exceeding the asci, 1.5–2 μm wide.

NOTES: The two specimens examined are basically the same in morphology, although the subhymenium, not distinguishable in the holotype, is well developed in the isotype, which we regard as infraspecific variation.

Among the known *Hymenoscyphus* species possessing stipitate apothecia and occurring on leaves, *H. yunnanicus* is most similar to *H. phyllogenus* (Rehm) Kuntze in excipular structure. The latter differs in white and much smaller (0.3–0.5 mm in diam.) apothecia, shorter (65–75 × 8–11 μm) asci, and ascospores that are smaller (11–16 × 4–6 μm), eguttulate and ellipsoid (Dennis 1956; White 1943).

Another foliicolous *Hymenoscyphus* species that possesses stipitate apothecia and fusoid multiguttulate ascospores is *H. crataegi* Baral & R. Galán from Spain on leaves of *Crataegus monogyna*. It differs from *H. yunnanicus* by

smaller apothecia (0.4–0.6 mm in diam.), slightly gelatinized ectal excipular tissue composed of much larger cells (12–43 × 10–16 μm), smaller asci (60–80 × 6–7.5 μm), and smaller ascospores [14–17.5 × (3–)3.5–4(–4.5) μm] (Baral et al. 2006).

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Gloeophyllum protractum is synonymous with *G. mexicanum*

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Abstract — *Gloeophyllum protractum*, a boreal polypore, is synonymized with the subtropical *G. mexicanum*. This taxonomic opinion is supported by sequencing of ITS and nuLSU regions. Morphology and ecology of this species are discussed.

Key words — *Basidiomycota*, *Gloeophyllales*, *Gloeophyllaceae*, molecular taxonomy

Introduction

The species of *Gloeophyllum* P. Karst. comprise a distinct group among polypores distinguished by a brown rot, tough dark brown context, and very variable (poroid, daedaleoid, or lamellate) hymenophore (Gilbertson & Ryvarden 1986). The six species (*G. abietinum* (Bull.) P. Karst., *G. carbonarium* (Berk. & M.A. Curtis) Ryvarden, *G. odoratum* (Wulfen) Imazeki, *G. protractum* (Fr.) Imazeki, *G. sepiarium* (Wulfen) P. Karst., and *G. trabeum* (Pers.) Murrill) known from Europe (Ryvarden & Gilbertson 1993, Niemelä 2005) are differentiated based on some critical morphological features and ecology, because the hyphae, hymenium, and spores are very similar in all species. In North America, these same taxa occur together with *G. mexicanum* (Mont.) Ryvarden (mostly on pines in subtropical regions and with *G. striatum* (Sw.) Murrill (a hardwood-specific, tropical species). Of the eight species found in East Asia (Dai 2012), seven are also found in Europe and North America and one (*G. imponens* (Ces.) Teng) is a tropical species growing on angiosperm wood (Dai et al. 2011, Dai 2012). All European species represent very common wood-inhabiting fungi except for *G. protractum*, which is very rare in Europe, limited to inner Scandinavia (Ryvarden & Gilbertson 1993) and some recently described localities in the Mediterranean region (Bernicchia et al. 2007, Tura et

al. 2010). In contrast, in the USA *G. protractum* is described as widespread from Florida to Alaska (Gilbertson & Ryvarden 1986) and is occasionally recorded also in East Asia (Dai et al. 2004, Dai 2012).

The unique phylogenetic position of the *Gloeophyllum* clade, separated from other polypores, was extensively studied by Garcia-Sandoval et al. (2011). Although many *Gloeophyllum* spp. sequences have been published, *G. protractum* is represented in GenBank by only one sequence, which is, however, identical with *G. odoratum* sequences. To clarify this issue, we sequenced variable rRNA regions of typical specimens of *G. odoratum*, *G. protractum*, and *G. mexicanum*.

Materials & methods

Specimens of *Gloeophyllum* were collected by the authors in Europe and USA or obtained from University of Helsinki herbarium, Finland (H). Our collected specimens are deposited in private herbarium of J. Vlasák (JV); some duplicates were also deposited in Prague Museum Herbarium (PRM).

Specimens examined: ***Gloeophyllum carbonarium***: **USA**, **Florida**, Everglades NP, pine, 29.VIII.2010, leg. & det. J. Vlasák (JV1008/67; GenBank JX266860, JX266862); Mahagony Hammock, pine, 21.XII.2003, leg. J. Vlasák Jr., det. J. Vlasák (JV0312/21.10-J; GenBank JX266861, JX266863).

Gloeophyllum mexicanum: **USA**, **Florida**, Everglades NP, pine, 29.VIII.2010, leg. & det. J. Vlasák (JV1008/67; GenBank JX266857); Big Cypress Nat. Preserve, Collier-Seminole State Park, 25.XII.2003, leg. J. Vlasák Jr., det. J. Vlasák (JV0312/25.14-J); Ocala, Ocala Nat. Forest, pine, 19.XII.2002, leg. J. Vlasák Jr., det. J. Vlasák (JV0212/35-J, PRM 860472; GenBank JX266856, JX266864).

Gloeophyllum odoratum: **CZECH REPUBLIC**, Hluboká nad Vltavou, Borek, spruce stump, 2.XI.2004, leg. & det. J. Vlasák (JV0411/2; GenBank JX266858, JX266859).

Gloeophyllum protractum: **USA**, **California**, Sierra Nevada, Tioga Pass, lodgepole pine, 18.VIII.2001, leg. & det. J. Vlasák (JV0108/100, PRM 860473; and JV 0108/101, PRM 860474; GenBank JX266850, JX266865 [sequences of both specimens identical]); **Oregon**, Crater Lake, pine, 15.IX.2007, leg. J. Vlasák Jr., det. J. Vlasák (JV 0709/135-J; GenBank JX266851, JX266852). **FINLAND**, **Inarin Lappi**, Inari, Lemmenjoki National Park, fallen charred trunk of *Pinus sylvestris*, 1.IX.1974, leg. & det. T. Niemelä (H 6012949; GenBank JX266853, JX266854); fallen decorticated trunk in dry site, 3.IX.1980, leg. & det. T. Niemelä (H 6012948; GenBank JX266855).

DNA methods

DNA was isolated and sequenced as described by Vlasák & Kout (2011). The evolutionary history was inferred by using the Maximum Likelihood method; the analyses were conducted in MEGA 5 (Tamura et al. 2011).

Results & discussion

We sequenced rRNA ITS region of several *Gloeophyllum mexicanum* and *G. protractum* specimens and one typical *G. odoratum* specimen. To root the phylogeny we used our sequences of *G. carbonarium* (considered closely related to *G. odoratum* by Garcia-Sandoval et al. 2011). Other *G. carbonarium*,

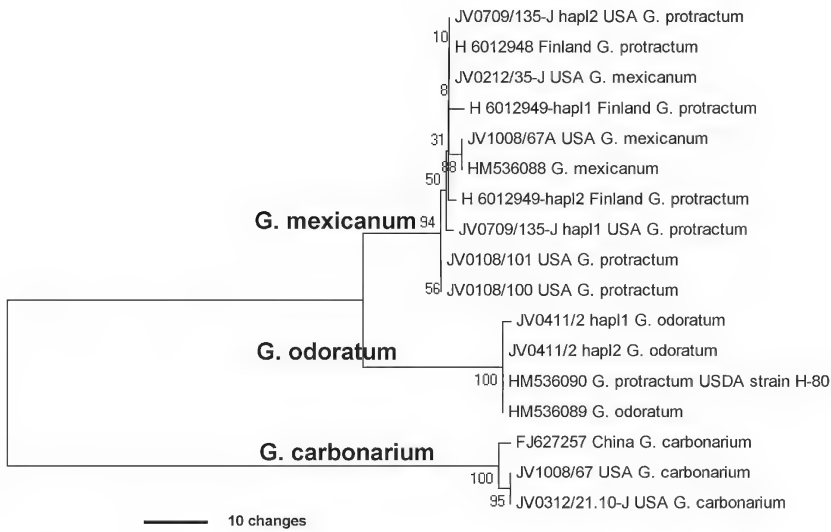

FIG. 1: Phylogenetic relationships of *Gloeophyllum* species inferred with ITS rRNA sequences. Sequenced specimens were determined according to their morphology; other sequences were retrieved with their names from GenBank. *Gloeophyllum carbonarium* was used to root the tree. Topology from maximum likelihood analysis. Support values along branches from maximum likelihood bootstrap. Branch lengths are drawn proportional to genetic distances (bar indicates 10 changes per ITS1-5.8S-ITS2 region). Some of the specimens show two haplotypes differing by short insertions/deletions. GenBank numbers indicate sequences retrieved from GenBank; other GenBank numbers in Materials & method.

G. mexicanum, and *G. odoratum* sequences were retrieved from GenBank. Alignment and simple analysis showed that the *G. mexicanum* sequences are identical with *G. protractum* sequences (FIG. 1). In particular, the *G. protractum* and *G. mexicanum* ITS sequences differ on average in only two positions, which corresponds to differences between individual *G. mexicanum* specimens or between different haplotypes in one collection. On the other hand, in *G. odoratum* approximately 10% of the positions differ from *G. mexicanum/protractum* sequences. The same result — identity of *G. mexicanum* with *G. protractum* and a pronounced divergence in *G. odoratum* — was obtained using nuLSU sequence analysis (not shown). The ITS2 region in one of two European *G. protractum* specimens had a unique insertion (4b) not present in the other specimen from the same locality. Accordingly, identity of European and American *G. mexicanum/protractum* specimens was also confirmed.

The name *Gloeophyllum mexicanum* [originally proposed in 1843 from Mexico as *Lenzites mexicana* Mont. (Montagne 1843)] has priority

Fig. 2: *Gloeophyllum mexicanum* JV0212/35-J, Florida, typical, photo in collection.

over *G. protractum* [originally described in 1851 from northern Europe as *Trametes protracta* Fr. (Fries 1851)]. Gilbertson & Ryvarden (1986) separate *G. mexicanum* (Fig. 2) from *G. protractum* based primarily on the daedaleoid to lamellate hymenophore (which they describe as poroid to radially elongated in *G. protractum*). Nevertheless, in our collections both from California (Figs 3, 4) and Finland, one specimen had a more or less poroid hymenophore while the other from the same locality showed a daedaleoid to lamellate structure. We infer that this feature is not diagnostic. In other respects — especially in the glabrous to semi-glossy pileal surface unique in the genus as well as microscopically — the two specimens appear identical. Particularly, *G. mexicanum* is described as pileate polypore with sessile and broadly attached fruitbodies elongated along the substrate, 3–10 × 1–3 cm, ≤1 cm thick, mostly applanate, more rarely triquetrous, with upper surface at first finely tomentose but soon glabrous with a poroid to daedaleoid hymenophore, occasionally with few lamellae 8–12 per cm, trimitic hyphal system, and basidiospores 9–12 × 3.5–4 µm; *G. protractum* is described as a pileate polypore with sessile fruitbodies distinctly elongated along the substrate, 10 × 4 cm, 5–15 mm thick, often slightly triquetrous in section, with a glabrous to semi-glossy pileal surface when juvenile, pores entire to weakly elongated radially, 1–2 per mm, trimitic hyphal system, and basidiospores 8.5–11(–12) × 3–4(–4.5) µm (Gilbertson & Ryvarden 1986). The main difference is their ecology, with *G. mexicanum* regarded as a subtropical or tropical American species and *G. protractum* as boreal or alpine in Europe where it was described. In the USA, we also found the typically thick poroid *G. protractum* only in the Sierra Nevada at 3000 m a.s.l., not at lower elevations. But we believe that as it descends the fungus becomes more applanate and lamellate so that it forms a "typical" *G. mexicanum* fructification near the

Fig. 3: *Gloeophyllum mexicanum* JV0108/101, Sierra Nevada, Tioga Pass, determined originally as *G. protractum*, photo in situ.

Fig. 4: *Gloeophyllum mexicanum* JV0108/100, Sierra Nevada, Tioga Pass, determined originally as *G. protractum*, photo in collection.

southern USA border. Intermediate forms may be sometimes determined as *G. mexicanum*, sometimes as *G. protractum*, and this is the reason, in our opinion, why so many *G. protractum* finds are reported from lowland and southern USA (Gilbertson & Ryvarden 1986).

From the above descriptions, inspection of our own specimens, and molecular data we infer that the crucial features of *G. mexicanum* and *G. protractum* are in total agreement. We show that different ecology and associated differences in morphology need not result in species differentiation as now understood (Taylor 2000). Similar results were recently obtained with very variable American specimens of *Fuscoporia viticola* (Schwein.) Murrill analyzed by molecular methods (Vlasák et al., unpublished).

There is something odd also with two other *Gloeophyllum* species recorded from the USA: *G. odoratum* and *G. abietinum*. These extremely common European fungi are reported from only rather limited areas in the western USA (Gilbertson & Ryvarden 1986) in a region known for its phytogeographical uniqueness. We regard the distribution maps of *G. odoratum* as unreliable because *G. protractum* has probably been misidentified as *G. odoratum* in the USA. We were not able to find either species when we visited the putative areas of *G. odoratum* and *G. abietinum* occurrence in the USA.

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Pseudofusicoccum adansoniae isolated as an endophyte from *Jatropha podagrica:* new record for India

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Abstract — *Pseudofusicoccum adansoniae* (*Botryosphaeriaceae*), representing a new generic record for India, is described and illustrated. The species, isolated as an endophyte from healthy leaf midrib and fruit of *Jatropha podagrica,* is characterized by large conidiomata and hyaline ellipsoid conidia with round apices. Its teleomorph is unknown. Comparison of the internal transcribed spacer 1 and 2 loci and 5.8S rDNA sequences showed 100% sequence similarity with that of CBS 122055, the ex-type strain of *P. adansoniae.*

Key words — anamorph, *Botryosphaeriaceae*, ITS, phylogeny, taxonomy

Introduction

Pseudofusicoccum Mohali et al. (*Botryosphaeriaceae*) is a relatively new genus typified by *Pseudofusicoccum stromaticum* (Mohali et al.) Mohali et al. (Crous et al. 2006). Recent morphological studies and ITS1-5.8S-ITS2 sequence analyses indicate that *Pseudofusicoccum* species occur in Venezuela and Brazil (*P. stromaticum*), Western Australia (*P. adansoniae* Pavlic et al., *P. ardesiacum* Pavlic et al., *P. kimberleyense* Pavlic et al.), and South Africa (*P. olivaceum* J.W.M. Mehl & B. Slippers, *P. violaceum* J.W.M. Mehl & B. Slippers) (Mohali et al. 2006, 2007; Pavlic et al. 2008; Mehl et al. 2011; Marques et al. 2012) (Table 1). Although some species have been isolated from dead wood, most have been isolated from healthy plant tissues where these fungi occur seemingly as endophytes. Recently, during an investigation of endophytic fungal diversity of *Jatropha podagrica* Hook. (*Euphorbiaceae*), two isolates were recovered with the morphological characteristics of *Pseudofusicoccum adansoniae.* The plant is considered toxic (Ojewole & Odebiyi 1980) and used for tanning leather and the production of a red dye (Bassam 2010). A literature survey indicated

that *Pseudofusicoccum* has not previously reported from India (Bilgrami et al. 1991, Jamaluddin et al. 2004), and *P. adansoniae* has been reported only from Australia. The present communication describes and illustrates these isolates in light of the molecular sequence data from ITS rDNA loci.

Materials & methods

The fungus was isolated from healthy leaf midrib and fruit of the perennial plant *Jatropha podagrica* (Buddha belly or Gout plant) from Pune (Pimpri), Maharashtra, India (18°37′07.04″N 73°48′13.43″E). Plant parts were washed in running tap water, sterile distilled water (3 washes) and cut into fragments (1–2 cm). Pieces of the plant were dipped and washed with 75% ethanol (5 min, twice) and 100% ethanol (30 sec), placed on Potato Dextrose agar (PDA) (Hi-Media, India) and Czapek Dox agar (CDA) (Hi-Media, India) media, and incubated at room temperatures (RT; c. 28–30 °C). After 4–5 days, mycelial growth was observed on the CDA Petri dish. The cultures were purified on 2% Malt Extract agar (MEA) plates and strains designated as MMI00062 (from leaf midrib) and MMI00064 (from fruit). Unstained microscopic observations were made using lactophenol (Hi-Media, India) mounts and observed under a Nikon YS100 microscope (Nikon, Japan). Measurements of morpho-taxonomic characters were recorded and compared with type descriptions of known species (Mohali et al. 2006, Pavlic et al. 2008, Mehl et al. 2011). Rayner's colour chart (1970) was used as a reference for culture colours.

Scanning Electron Microscopy (SEM) of air-dried pycnidia was done by coating with platinum in Jeol sputter coater (JFC 1600) and examining on a Jeol-JSM 6360A SEM at 10 kV. The culture MMI00064 has been deposited in the Microbial Culture Collection (WDCM-930), Pune, India as MCC 1020.

For sequencing of the ITS loci, DNA was extracted from pure cultures grown in Petri dishes using the QIAamp® DNA Mini Kit (Qiagen, Inc., Valencia CA). DNA concentration was estimated using a Nanodrop ND-1000 machine (Thermo scientific, USA). The genomic DNA was amplified using ITS1 and ITS4 primers (White et al. 1990) in a PE 9700 thermocycler (PE, Applied Biosystems, Singapore) with the following conditions: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The positive amplicons were purified using a PCR Cleanup Kit (Qiagen, Inc., Valencia CA) and purified PCR products were sequenced (both strands) on an ABI 3730 xl DNA analyzer using the Big Dye terminator kit (Applied Biosystems, Inc., Foster City, CA). An NCBI BLASTn search was conducted for sequence similarity (Zhang et al. 2000).

For phylogenetic relationships with *Pseudofusicoccum adansoniae* MCC 1020, 11 related sequences were retrieved from GenBank database. *Neoscytalidium novaehollandiae* CBS 122071 (EF585540.1) was used as the outgroup and sequence alignment carried out using MUSCLE (Edgar 2004). Maximum parsimony (MP) analysis and phylogenetic tree construction was conducted using MEGA 5.0 (Tamura et al. 2011).

Table 1: Morphological comparison of *Pseudofusicoccum adansoniae* MCC 1020, *P. adansoniae* CBS 122055, and other species.

	P. adansoniae MCC 1020	*P. adansoniae* Pavlic et al. 2008	*P. kimberleyense* Pavlic et al. 2008	*P. ardesiacum* Pavlic et al. 2008	*P. violaceum* Mehl et al. 2011	*P. olivaceum* Mehl et al. 2011	*P. stromaticum* Mohali et al. 2006
Colony diam.	90 mm	90 mm	90 mm	90 mm	—	—	70–75 mm
Colony colour	Gray olivaceous to olivaceous black	Gray olivaceous to olivaceous black	Olivaceous black to black	Violaceous gray to slate blue	Olivaceous to greenish black	White to olivaceous	—
Optimum temp.	28–30°C	30°C	30°C	30°C	30°C	25°C	—
Pycnidia	200–500 µm (in culture)	500 µm (on host)	510 µm (on host)	500 µm (on host)	500–620 µm (on host)	530–630 µm (on host)	—
Conidia (mean, in µm)	Ellipsoid, occas. bent or irreg. shaped (16.5 × 7.3)	Apices rounded, smooth with fine granular content (22.5 × 5.2)	Ellipsoid, straight / slightly curved (30.7 × 7.4)	Ellipsoid to rod-shaped, straight / sl. bent (25 × 7.5)	Bacilliform, granular, guttulate (33.0 × 9.5)	Guttulate (22.8 × 7.0)	Bacilliform (21.7 × 5.4)
Conidiogenous cells (mean, in µm)	Cylindrical (10.2 × 2.0)	Cylindrical (12.7 × 2.4)	(Sub)cylindrical (9.8 × 3.3)	Cylindrical (8.6 × 3.5)	Cylindrical (8.6 × 4.3)	Cylindrical-guttulate-circular (6.6 × 3.7)	Cylindrical (13 × 2.5)
% Similarity with MCC 1020	—	100% (0 nt)	98.8% (6 nt)	98.8% (6 nt)	98.6% (7 nt)	98% (10 nt)	98.4% (8 nt)
Hosts	*Jatropha podagrica*	*Adansonia gregorii, Acacia synchronicia, Eucalyptus* sp., *Ficus opposita*	*A. gregorii, Ac. synchronicia, Eucalyptus* sp., *F. opposita*	*A. gregorii, Eucalyptus* sp.	*Pterocarpus angolensis*	*Pt. angolensis*	*Eucalyptus* sp.
Distribution	India	Australia	Australia	Australia	South Africa	South Africa	Venezuela

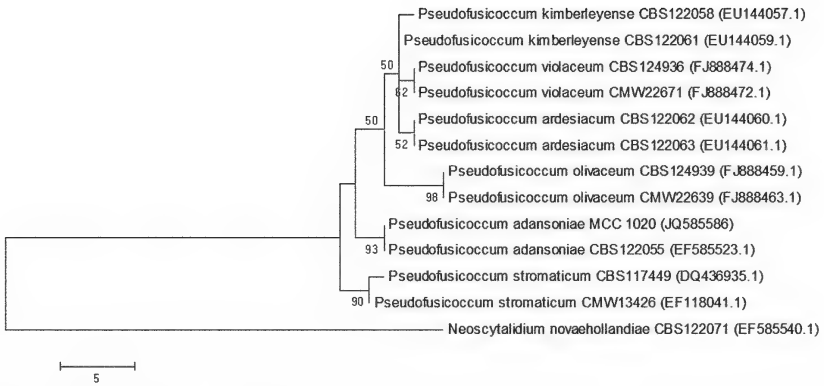


Plate 1. Maximum parsimony tree (by complete deletion of gaps) based on ITS sequences (ITS1-5.8S-ITS2) depicting the position of *P. adansoniae* MCC 1020 within *Pseudofusicoccum* and its relation with other closely related phylospecies. Bootstrap values (1000 replicates) shown above branches.

Results

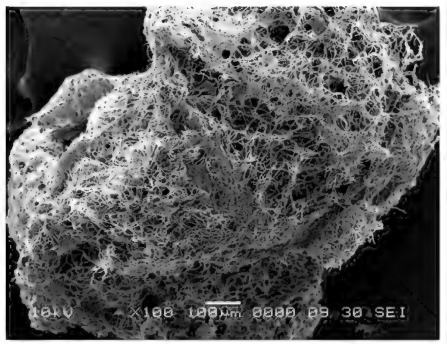
The cultures isolated were identified as *Pseudofusicoccum adansoniae* based on colony size and colour, conidial size, and conidiophore morphology as described by Pavlic et al. (2008). When the ITS sequence data of six *Pseudofusicoccum* species procured from GenBank were aligned with our isolate (JQ585586) sequence, 44 ambiguous base pairs bp were removed from the total 568 (bp) prior to analysis. Of the 524 bp used to reconstruct the phylogeny, 457 were conserved, 64 were variable, 16 were parsimony-informative, and 48 were singletons. Maximum parsimony analysis produced eleven equally parsimonious trees with a tree length of 68 (CI = 0.941176, RI = 0.857143, and RCI = 0.806723 for all sites). One such most parsimonious tree (Plate 1) exhibits three major clades: Clade 1 (*P. kimberleyense, P. violaceum, P. ardesiacum*), Clade 2 (*P. stromaticum*, *P. adansoniae* MCC 1020, *P. adansoniae* CBS 122055), and Clade 3 (consisting of *P. olivaceum*). The Maximum parsimony (MP) tree showed *P. adansoniae* MCC 1020 forming a separate clade along with *P. adansoniae* CBS 122055, but forming a sister branch to *P. stromaticum*. Moreover, the strain MCC 1020 and *P. adansoniae* CBS 122055 showed 100% similarity within 507nt of the ITS region when compared in MEGA 5.0 (Tamura et al. 2011).

Taxonomy

Pseudofusicoccum adansoniae Pavlic, T.I. Burgess & M.J. Wingf., Mycologia 100: 855. 2008. Plate 2

Teleomorph: Unknown.

Colonies initially form a moderately dense mycelial mat. Initially mycelium pale olivaceous grey (21′′′′′d) to olivaceous grey (21′′′′′b) from the middle of



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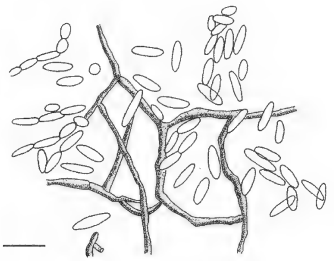

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PLATE 2. *Pseudofusicoccum adansoniae*. FIG. 1. Conidiomata detail showing mycelial hairs covering the surface (SEM). FIG. 2. Conidia. Scale bar = 25µm.

the colony. At maturity, grey olivaceous (21′′′′i) to olivaceous black (27′′′′m) and after 3–5 d becoming dark greenish-black (33′′′′′k) with age, covering the 90 mm diam Petri dish after 4 d in the dark on 2% MEA medium. Colonies slightly irregular, occasionally radially striated with zones or irregular circles. Aerial mycelium slightly fluffy, becoming dense and cottony with age, turning pale olivaceous grey (21′′′′′d). Radiations are evident on front and reverse of colony. Conidiomata 1–2 mm, some 5 mm in diam, readily formed from the middle of colony within 7–10 d, covering the entire surface of colony and deeply immersed in the medium (seen as a round black structures on the reverse side of Petri dishes 14–16 d after incubation), eustromatic, multilocular, immersed. Hyphae brown, branched. Conidiogenous cells holoblastic, smooth, cylindrical, hyaline, the first conidium produced holoblastically and subsequent conidia enteroblastically. Conidia ellipsoid, occasionally slightly bent or irregularly shaped, 9.7–25.8 × 6.5–9.7 µm (av. 16.5 × 7.3 µm), apices rounded, smooth with fine granular content, hyaline, thin-walled, unicellular, surrounded by a mucous layer.

DISTRIBUTION AND HABITAT: Western Australia (from infected *Adansonia gregorii* and *Mangifera indica*, and from asymptomatic *Acacia synchronicia*, *A. gregorii*, *Eucalyptus* sp., and *Ficus opposita*); Maharashtra, India (endophytic in *Jatropha podagrica*).

MATERIAL EXAMINED: **INDIA**, **MAHARASHTRA STATE**, Pune, Pimpri, 18°37′07.04″N 73°48′13.43″E, endophytic in leaf midrib of *Jatropha podagrica*, 25.IV.2011, Rohit Sharma, culture MMI00062; endophytic in fruit of *J. podagrica*, 25.IV.2011, Rohit Sharma, culture MMI00064 (MCC 1020; GenBank JQ585586).

Discussion

Pseudofusicoccum adansoniae MCC 1020 is characterized by multilocular hairy conidiomata with several pycnidia whose inner margin is covered with hyaline mycelia. The fungus produces several conidiomata (10–15) in

culture covered by mycelial hairs (Fig. 1). It contains several hyaline, ellipsoid conidia (Fig. 2) formed on hyaline conidiophores covered in a mucous layer. However, the conidia of *P. adansoniae* MCC 1020 average 16.5 × 7.3 µm (Table 1) and are narrower and broader compared to the average 22.5 × 5.2 µm of *P. adansoniae* CBS 122055 (Pavlic et al. 2008). Table 1 clearly shows the similarity of a combination of characters (e.g., colony colour, growth characters, conidiogenous cell and conidial morphologies) for MCC 1020 and the other analyzed *P. adansoniae* strain. Phylogenetic analysis suggests that *P. adansoniae* is only distantly related to all other *Pseudofusicoccum* species. ITS1-5.8S-ITS2 sequence analyses place *P. adansoniae* MCC 1020 and *P. adansoniae* CBS 122055 together in a distinct clade forming a sister branch to *P. stromaticum*. The maximum parsimony analysis and resulting tree (Plate 1) clearly indicates that apart from the *P. adansoniae* and *P. stromaticum* branches, *Pseudofusicoccum* is divided into two major groups, one containing *P. violaceum, P. ardesiacum,* and *P. kimberleyense* and the other containing *P. olivaceum*. Neighbour Joining analysis also generates similar tree topologies.

Most species (including *Pseudofusicoccum*) of the *Botryosphaeriaceae* are endophytic, and some can be latent pathogens (Crous et al. 2006, Slippers & Wingfield 2007). *Pseudofusicoccum adansoniae* CBS 122055 was originally described from Derby, Western Australia, and isolated from infected branches of *Adansonia gregorii* F. Muell. (=*Adansonia gibbosa* Cunn. et al.) (*Malvaceae*) and healthy branches of *Acacia synchronicia* Maslin (*Leguminosae*), *Eucalyptus* sp., and *Ficus opposita* Miq. (*Moraceae*) (Pavlic et al. 2008). The fungus has also been isolated as an endophyte from *Adansonia gregorii* (Sakaladis et al. 2011a) and from diseased stems of *Mangifera indica* L. (*Anacardiaceae*) from Western Australia (Sakaladis et al. 2011b). Hence, detection of *P. adansoniae* MCC 1020 from healthy leaf midrib and fruit of *Jatropha podagrica* is not unusual, as it has been isolated from asymptomatic branches of other trees. As it represents a first species record outside Australia and the first *Pseudofusicoccum* record from India, it is reported as new to the Indian fungal flora. Reports of *Pseudofusicoccum* from other parts of world — especially as endophytes — should be expected.

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Pestalotiopsis species associated with *Camellia sinensis* (tea)

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Abstract — We describe a new species *Pestalotiopsis furcata* isolated from *Camellia sinensis* (tea), which is distinguished morphologically by its relatively large conidia (29–39 × 8.5–10.5 µm) and 5–9 apical appendages, some of which are branched and lack basal appendages. A phylogenetic tree based on a combination of ITS, β-tubulin and TEF1 clearly distinguishes *P. furcata* from other species in the genus. We examined syntype material of *P. theae,* which we designate as lectotype, and a fresh collection from Thailand, which we designate as epitype.

Key words — *Amphisphaeriaceae,* grey blight, phylogeny, systematics, tea disease

Introduction

Camellia sinensis (L.) Kuntze (*Theaceae*) is widely planted in the tropics and subtropics and probably originated at the point of confluence of the lands of northeast India, north Burma, southwest China and Tibet (Wight 1959; Mondal et al. 2004). Commonly known as tea, it is prepared as a beverage with a cooling, slightly bitter, astringent flavor and is the most widely consumed liquid in the world after water (Mondal et al. 2004). Several fungi are known to cause diseases of foliage, stems and roots of *C. sinensis*. Brown blight (*Colletotrichum camelliae* Massee), leaf blotch (*Colletotrichum* sp.), grey blight (*Pestalotiopsis longiseta* (Speg.) K. Dai & Tak. Kobay. and *P. theae*, blister blight (*Exobasidium vexans* Massee), twig die-back and stem canker (*Macrophoma theicola* Petch), and horse hair blight (*Marasmius crinis-equi* F. Muell. ex Kalchbr.) are common fungal diseases affecting tea plantations (Gadd 1949; Hainsworth 1952; Chen et al. 1982).

The genus *Pestalotiopsis* contains several species responsible for plant diseases. *Pestalotiopsis* species have been isolated as both endophytes and pathogens in *Camellia sinensis* (Wei et al. 2007; Joshi et al. 2009). *Pestalotiopsis* is an anamorphic genus in *Amphisphaeriaceae* (Barr 1975, 1990; Kang et al. 1998, 1999; Maharachchikumbura et al. 2011, 2012). In southern India grey blight disease of tea caused by *Pestalotiopsis* spp. resulted in 17% production loss (Joshi et al. 2009) and 10–20% yield loss in Japan (Horikawa 1986). Five *Pestalotiopsis* species have been recorded from tea (Agnihothrudu 1964), although *P. longiseta* and *P. theae* are considered to be the major species causing grey blight (Joshi et al. 2009).

The taxonomic status of species in *Pestalotiopsis* is unresolved, as many species are generally not host-specific, conidial characters vary, and species limits overlap. The species concepts adopted by Steyaert (1949) and Guba (1961) are problematic (Maharachchikumbura et al. 2011). Epitypification of important species using molecular data and analyses is needed to resolve the species and to establish their important diagnostic characters, as has been done for several other plant pathogenic genera such as *Colletotrichum* (Hyde et al. 2009), *Phomopsis (*Udayanga et al. 2011), *Cochliobolus* (Manamgoda et al. 2011), and *Phyllosticta* (Wikee et al. 2011). Re-examination of type material and establishment of epitypes from fresh collections relying on sequence analyses from living cultures and full descriptions are needed to resolve species in *Pestalotiopsis* and other important pathogenic genera (Hyde et al. 2010; Cai et al. 2011; Koko et al. 2011).

In order to re-evaluate *Pestalotiopsis* species associated with tea (*C. sinensis*), we examined syntype material of *P. theae*, which we here describe, illustrate, and designate as lectotype. We also epitypify this economically important taxon with a fresh collection from tea in Thailand and fully describe the new collection. Sequence data from the epitype allows us to distinguish *P. theae* from other *Pestalotiopsis* collections from tea. We also describe *P. furcata,* a new *Pestalotiopsis* species from tea in Thailand, which is differentiated by morphological and molecular differences.

Materials and methods

Study site and plant material

Diseased leaves of tea were collected from Chiang Mai Province, northern Thailand, near the Mushroom Research Centre, at an elevation of 900 m at 19°17.123′N 98°44.009′E. The region has a mean annual temperature of 24°C and mean annual precipitation of 2121 mm (http://www.tmd.go.th/en/). Leaf samples were placed in clean polythene bags and symptoms were recorded.

Isolation and identification of pathogen

A single conidium culture technique was used to obtain pure fungal colonies according to Chomnunti et al. (2011). The colonies were transferred to 2% potato-

dextrose agar (PDA) medium and incubated at room temperature (25°C). Sporulation was induced on sterilized carnation leaves that were aseptically placed on the surface of PDA with growing mycelium. The morphology of fungal colonies was recorded following the method of Hu et al. (2007). Fungal mycelium and spores were observed under the light microscope and photographed. Microtome sections were made with a Leica CM1850 freezing microtome. All the microscopic measurements were measured with Tarosoft image framework (v. 0.9.0.7) and 30 conidial measurements were taken for each isolates.

All other cultures were obtained from China General Microbiological Culture Collection (CGMCC) and a syntype herbarium specimen of *P. theae* (BPI 406804) was borrowed from BPI and studied. Methods of examination, photography, and isolation followed Boonmee et al. (2011).

DNA extraction and PCR condition

Total genomic DNA was extracted from fresh cultures using a modified protocol of Doyle & Doyle (1987) and Lee & Taylor (1990). Fresh fungal mycelia (500 mg) was scraped from the margin of a PDA plate incubated at 25°C for 7 to 10 days and transferred into a 1.5 mL centrifuge tube with 100 µL of preheated (60°C) 2× CTAB extraction buffer (2% (w/v) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0), and 200 mg sterilized quartz sand. Mycelia were ground using a glass pestle for 5 min and an extra 500 µL 2× CTAB preheated (60°C) was added and incubated in a 65°C water bath for 30 min with occasional shaking. 500 µL of phenol:chloroform (1:1) was added to each tube and shaken thoroughly to form an emulsion. The mixture was spun at 11900 *g* for 15 min at 25°C in a microcentrifuge and the supernatant phase decanted into a fresh 1.5 mL tube. Supernatant containing DNA was re-extracted with phenol: chloroform (1:1) at 4°C until no interface was visible. 50 µL of 5M KOAc was added into the supernatant followed by 400 µL of isopropanol and inverted gently to mix. The genomic DNA was precipitated at 9200 *g* for 2 min at 4°C in a microcentrifuge. The DNA pellet was washed with 70% ethanol twice and dried using SpeedVac® (AES 1010; Savant, Holbrook, NY, USA) until dry. The DNA pellet was then resuspended in 100 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR amplification

The ITS and 5.8S region of rDNA molecule was amplified using primer pairs ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (White et al. 1990), β-tubulin gene region was amplified with primer pairs BT2A (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and BT2B (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass & Donaldson 1995; O'Donnell & Cigelnik 1997) and TEF1 was amplified using the primer pairs EF1-526F (5′-GTCGTYGTYATYGGHCAYGT-3′) and EF1-1567R (5′-ACHGTRCCRATACCACCRATCTT-3′) (Rehner 2001).

PCR was performed with the 25 µL reaction system consisting of 19.75 µL of double distilled water, 2.5 µL of 10× Taq buffer with $MgCl_2$, 0.5 µL of dNTP (10 mM each), 0.5 µL of each primer (10 µM), 0.25 µL Taq DNA polymerase (5 U/µL), 1.0 µL of DNA template. The thermal cycling program was as follows:

For ITS an initial denaturing step of 95°C for 3 min, followed by 35 amplification cycles of 95°C for 30 s, 52°C for 45 s, and 72°C for 90 s, and a final extension step of 72°C

Table 1. *Pestalotiopsis* isolates (and *Seiridium* outgroup) considered in the phylogenetic study

	Isolate	GenBank accession number		
		ITS	β-tubulin	TEF1
P. cf. *algeriensis*	SD077	JQ683718	JQ683702	JQ683734
P. cf. *disseminata*	SD034	JQ683716	JQ683700	JQ683732
P. cf. *menezesiana*	SG064	JQ683719	JQ683703	JQ683735
	SD072	JQ683713	JQ683697	JQ683729
P. cf. *microspora*	SD056	JQ683722	JQ683706	JQ683738
P. cf. *versicolor*	SG100	JQ683712	JQ683696	JQ683728
	SD047	JQ683715	JQ683699	JQ683731
	SD091	JQ683714	JQ683698	JQ683730
	SD040	JQ683717	JQ683701	JQ683733
P. cf. *virgatula*	SD004	JQ683723	JQ683707	JQ683739
P. furcata (ex-holotype)	MFLUCC12-0054	JQ683724	JQ683708	JQ683740
P. hainanensis (ex-type)	—	GQ869902	—	—
P. jesteri (ex-type)	—	AF377282	—	—
P. kunmingensis (ex-type)	—	AY373376	—	—
P. pallidotheae (ex-type)	—	AB482220	—	—
P. theae (ex-epitype)	MFLUCC12-0055	JQ683727	JQ683711	JQ683743
	SC011	JQ683726	JQ683710	JQ683742
Pestalotiopsis sp.	SD012	JQ683720	JQ683704	JQ683736
	SD072	JQ683713	JQ683697	JQ683729
Seiridium sp.	SD096	JQ683725	JQ683709	JQ683741

for 10 min. For β-tubulin PCR conditions were an initial step of 3 min at 95°C, 35 cycles of 1 min at 94°C , 50 s at 55°C , and 1 min at 72°C, followed by 10 min at 72°C. For TEF1, an initial step of 5 min at 94°C, 10 cycles of 30 s at 94°C, 55 s at 63°C or 66°C (decreasing 1°C per cycle), 90 s at 72°C, plus 36 cycles of 30 s at 94°C, 55 s at 53°C or 56°C, 90 s at 72°C, followed by 7 min at 72°C. The PCR products were verified by staining with Goldview (Guangzhou Geneshun Biotech, China) on 1% agarose electrophoresis gels.

Phylogenetic analysis

DNAStar, SeqMan was used to obtain consensus sequences from sequences generated from β-tubulin and TEF1, forward and reverse primers. Combination sequence data obtained from three gene regions were aligned using CLUSTALX (v. 1.83) (Thompson et al. 1997). The sequences were manually adjusted using BioEdit (Hall 1999), to allow maximum alignment and maximum sequence similarity. A maximum parsimony analysis (MP) was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were set up to 5000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and log likelihood [–ln L] (HKY model) were calculated for trees generated under different optimality criteria. The robustness of the

most parsimonious trees was evaluated by 100 bootstrap replications resulting from maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino–Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether the trees inferred under different optimality criteria were significantly different. Trees were viewed in Treeview (Page 1996).

The same procedure was used for the ITS gene for the 16 *Pestalotiopsis* isolates and the type sequences of *P. hainanensis*, *P. jesteri*, *P. kunmingensis,* and *P. pallidotheae* were downloaded from GenBank.

Results

A phylogenetic tree was constructed using combined ITS, β-tubulin and TEF1 sequences of 16 isolates of *Pestalotiopsis,* with *Seiridium* sp. as the outgroup (TABLE 1). The aligned data matrix comprised 2019 characters of which 1490 were constant, 199 were variable and parsimony-uninformative, and 330 were parsimony-informative. The Kishino-Hasegawa (KH) test showed that the two trees generated from parsimonious analysis did not differ significantly (length = 768 steps, CI = 0.848, RI = 0.905, HI = 0.152, RC = 0.767). The aligned data matrix of ITS sequences consisted of 528 characters of which 395 were constant, 62 were variable and parsimony-uninformative, and 71 were parsimony-informative. Kishino-Hasegawa (KH) test showed length = 178 steps, CI = 0.826, RI = 0.913, HI = 0.174, RC = 0.754).

Taxonomy

Pestalotiopsis theae (Sawada) Steyaert, Bull. Jard. bot. État Brux. 19(3): 327 (1949)

FIGS 1, 2

≡ *Pestalotia theae* Sawada, Spec. Report Agric. Exp. Station Formosa 11: 113 (1915), as "*Pestalozzia*"

TYPE: Taiwan, Taipei [Taihokuchô, Rigyokutsu (Tanaka 1917)], on living leaves of *Camellia sinensis*, 13 July 1908, coll. Y. Fujikiro, det. K. Sawada (**Lectotype designated here**, BPI 406804). Thailand, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, 900 m, rainforest, on living leaves of *Camellia sinensis*, January 20, 2010, S.S.N. Maharachchikumbura St200110 (**Epitype designated here**, MFLU 12-0116; ex-epitype culture, MFLUCC 12-0055 = CPC 20281; GenBank, JQ683727, JQ683711, JQ683743).

LECTOTYPE

LEAF SPOTS initially brown on leaves of tea, becoming 1 cm in diam., and grey with brown margins when mature, or covering up to half of the leaf; dotted with acervuli. ACERVULI initially subepidermal, later erumpent, finally exposed. CONIDIOPHORES in clusters, simple, short, filiform, fugacious. CONIDIA fusiform, slightly constricted at septa, 4-septate, 24–28 × 6.6–8.3 μm (mean = 26.5 × 7.4 μm); basal cell obconic, hyaline, thin and smooth-walled, 4.3–5.6 μm long (mean = 5 μm); 3 median cells, with thick verruculose walls,

dark brown, septa and periclinal walls darker than the rest of the cell, together 15–20 µm long (mean = 18.5 µm) second cell from base 6.5–7 µm (mean = 6.8 µm); third cell 4.8-5.5 µm (mean = 5.3 µm; fourth cell 5.5–6.0 µm (mean = 5.7 µm); apical cell hyaline, conic to cylindrical 4.2–5.8 µm long (mean =

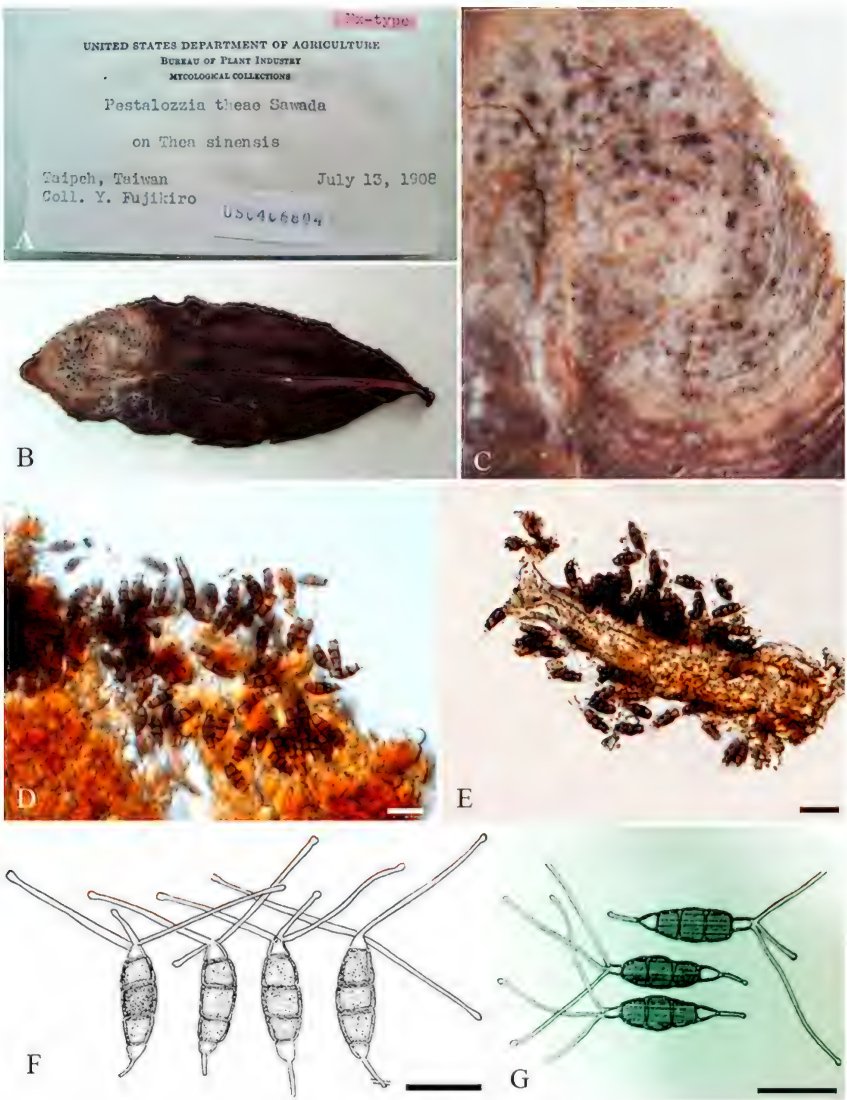

Fig. 1. *Pestalotiopsis theae* (lectotype, BPI 406804). A. Lectotype herbarium material. B,C. Leaf blight symptoms on leaf of *Camellia sinensis*. D,E. Conidia. F. Conidia (drawing from Steyaert 1943) G. Conidia (drawing from Guba 1961). Scale bars: D–G= 20 µm.

4.9 µm); apical appendages tubular, 3–4, arising from the upper portion of the apical cell, 23–33 µm long (mean = 27 µm), slightly swollen at the apex; basal appendages, filiform, 5–9 µm, sometimes knobbed.

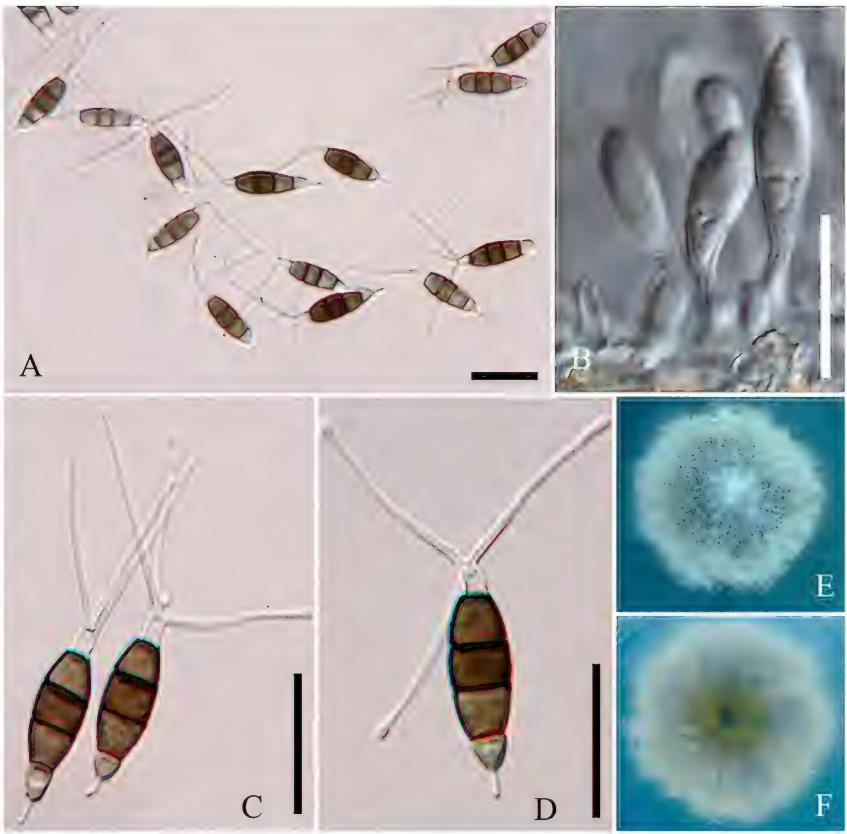

Fig. 2. *Pestalotiopsis theae* (epitype, MFLU 12-0116) A. Conidia in culture. B. Conidiogenous cells. C,D. Conidia. E,F. Colony in culture (E. from above; F. from below). Scale bars: A,B= 20 µm, C,D=15 µm.

Epitype

Conidiophores growing in clusters, simple, short, filiform, fugacious, smooth, thin-walled, hyaline, 4–8 × 1–2 µm (mean = 6 × 1.5 µm). Conidia fusiform to ellipsoid, straight to slightly curved, 4-septate 22.5–28 × 6.7–8.2 µm (mean = 25.5 × 7.6 µm), basal cell conic or obconic, hyaline, thin and smooth walled, 3.9–5.3 µm long (mean = 4.55 µm), with 3 median cells, thick verruculose walls, constricted at the septa, concolorous, dark brown, septa and periclinal walls darker than the rest of the cell, together 14.5–18.5 µm long

(mean = 16.7 µm) (second cell from base 5–7.2 µm (mean = 6.3 µm); third cell 4.8–6 µm (mean = 5.4 µm); fourth cell 5–6.8 µm (mean = 5.7 µm)); apical cell hyaline, cylindrical 4.2–5.9 µm long (mean = 5.2 µm); 3–4 apical appendages, tubular, arising from the upper portion of the apical cell, 22.5–31 µm long (mean = 26.5 µm), slightly swollen at the apex; basal appendages, filiform, 4–7 µm.

Colonies growing relatively fast on PDA, reaching 7 cm after 5 days at 25°C, fimbriate, whitish, dense, aerial mycelium on surface, fruiting bodies black; reverse of the culture yellowish white.

The syntypes of *P. theae* were recorded from diseased leaves of *Camellia sinensis* growing in Taiwan. The specimen from BPI corresponds with one of the collections listed in the translated protologue, and therefore constitutes a syntype specimen (Tanaka 1917, as "Taihokucho, Rigyokutsu, July 13, 1908, Y. Fujikuro"). Since no ex-type culture is available and the lectotype is in poor condition, an epitype with a living culture is designated from a sample collected in Thailand.

Pestalotiopsis furcata Maharachch. & K.D. Hyde, **sp. nov.** FIG. 3

MYCOBANK MB564563

Differs from other *Pestalotiopsis* species with large conidia by its wider conidia with 5–9 apical, branched appendages.

TYPE: Thailand, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, 20 January 2010, S.S.N. Maharachchikumbura S200110 (**Holotype**, MFLU 12-0112; ex-holotype culture MFLUCC 12-0054 = CPC 20280; GenBank, JQ683724, JQ683708, JQ683740).

ETYMOLOGY: The specific epithet refers to the branching nature of the apical appendages.

Associated with grey blight on leaves of *Camellia sinensis*, small, rounded, yellow-green spots on the leaves become brown to grey, with concentric rings bearing black, scattered conidiomata (FIG. 3). *Conidiomata* acervuli scattered or gregarious, rarely confluent, subepidermal in origin, erumpent when mature, round to oval in outline, conical to oval in longitudinal section, 180–300 µm wide, 70–160 µm high, unilocular, glabrous; wall tissue (stroma and parietal cells) only a few cells thick (14–22 µm), forming a textura angularis, cell walls thick, outermost layer hyaline, inner layers pale brown to brown, encrusted. *Conidiophores* reduced to conidiogenous cells lining the inner wall of the conidiomatal cavity. *Conidiogenous cells* discrete, lageniform, smooth, thin-walled, hyaline, with 2–3 proliferations. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, 29–39 × 8.5–10.5 µm (mean = 35.5 × 9.7 µm), basal cell obconic, hyaline or slightly olivaceous, thin- and smooth-walled, 4.9–6.4

Fig. 3. *Pestalotiopsis furcata* (holotype, MFLU 12-0112). A. Blight on leaf of *Camellia sinensis*. B. Conidiomata, split irregularly. C. Section of conidiomata. D. Conidiophores/conidiogenous cells. E–H. Conidia with branched appendages. I,J. Colony on PDA (I from above, J from below). Scale bars: C= 50 µm, D–H= 20 µm.

µm long (mean = 5.8 µm), with 3 median cells, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 20.7–25 µm long (mean = 23.4 µm) (second cell from base 7–9 µm (mean = 7.9 µm); third cell 7.5–9.1 µm (mean = 8.2 µm); fourth cell 7.2–9.2 µm (mean = 8.0 µm); apical cell hyaline, conic to cylindrical 6.3-8.44 µm long (mean = 7.48 µm); 5–9 tubular apical appendages, some appendages branched, arising from the upper portion of the apical cell, 20–35 µm long (mean = 27.7 µm), unequal; basal appendages absent.

Colonies on PDA reaching 7 cm after 7 days at 25°C, edge entire, whitish, with dense, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture white.

HABITAT/DISTRIBUTION: Known to inhabit living leaves of *Camellia sinensis*, Thailand.

ADDITIONAL MATERIAL EXAMINED: **THAILAND, CHIANG MAI PROV.,** MAE TAENG DISTR., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, 10 July 2010, S.C. Karunarathna S100710 (MFLU12-0113); 9 September 2011, S.S.N. Maharachchikumbura S110911 (MFLU12-0114); 9 December 2011, S.S.N. Maharachchikumbura S91211 (MFLU12-0115).

TABLE 2. Synopsis of *Pestalotiopsis furcata* and related species.

	P. furcata	*P. nattrassii* [a]	*P. leucopogonis* [b]	*P. macrospora* [a]	*P. hainanensi* [c]
Conidia size (μm)	29–39 × 8.5–10.5	27–33 × 8–9	27–32 × 7.5–9.5	30–40 × 7–9	19–22 × 5–6
Median cells	Concolorous, olivaceous	Concolorous, brown	Concolorous, brown	Concolorous, olivaceous	Concolorous, Brown to olivaceous
Apical appendages					
–number	5–9	1–4	7–11	3–5	1–3
–length (μm)	20–35	25–44	12–19	15–22	1–10
–branching	Branched	No	No	Branched	No
–position	Apex	Apex	3 rows (top, middle, and bottom)	Apex	Apex
Basal appendages	Lacking	Lacking or short	Lacking or present	Present	Lacking

[a] = from Guba (1961); [b] = from Nag Raj (1993); [c] = from Liu (2007)

NOTES: *Pestalotiopsis furcata* is both morphologically (TABLE 2) and phylogenetically (FIGS. 4–5) distinct. Its conidia (29–39 × 8.5–10.5 μm) are larger than those of other *Pestalotiopsis* species as well as wider than such morphologically similar species as *P. hainanensis* (19–22 × 5–6 μm), *P. leucopogonis* (27–32 × 7.5–9.5 μm), *P. macrospora* (30–40 × 7–9 μm), and *P. nattrassii* (27–33 × 8–9 μm). Its most characteristic feature is its 5–9 apical, branched appendages. Although *P. leucopogonis* also has 7–11 apical appendages, they arise from three levels attached to the apical cell (apex, middle, and base) and at 12–19 μm long are shorter than those of *P. furcata* (20–35 μm). *Pestalotiopsis macrospora* has shorter (15–22 μm) branched apical appendages. *Pestalotiopsis furcata* also lacks basal appendages, which are otherwise present in most *Pestalotiopsis* species.

Discussion

In the combined dataset *Pestalotiopsis* species cluster in two main clades with high (100%) bootstrap support (FIG. 5). These two clades can be

differentiated by the color of the median cells, confirming earlier reports (Jeewon et al. 2003; Liu et al. 2010; Maharachchikumbura et al. 2011, 2012). In the multigene analysis (Fig. 5), clade A (*P. theae*) was separate from clade B with high bootstrap support. When additional ITS sequences downloaded from GenBank were analyzed (results not shown), putatively named *P. theae* strains clustered in both clades, indicating that many have misapplied names. The median conidial cells of clade B strains are olivaceous, while clade A strains have brown median conidial cells similar to the type (Fig. 5). We blasted the

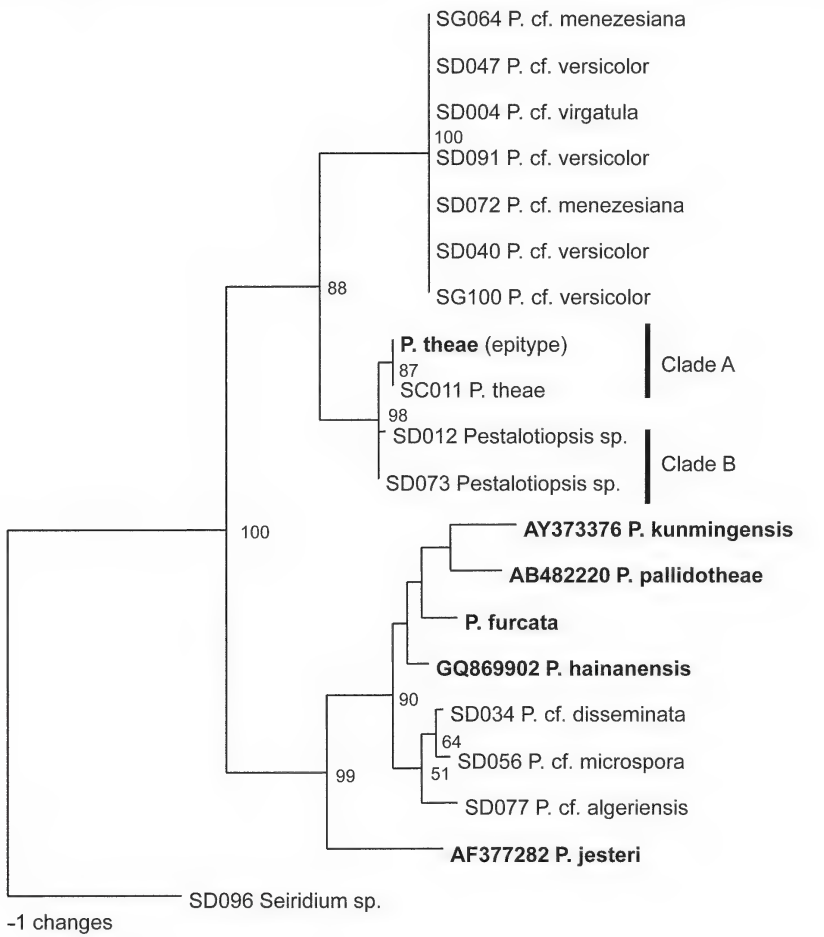

Fig. 4. Maximum parsimony phylogram generated from ITS gene region analysis of species of *Pestalotiopsis* recorded from tea and other available *Pestalotiopsis* spp. type sequences. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* sp. is placed as outgroup, and all ex-type sequences are set in bold font.

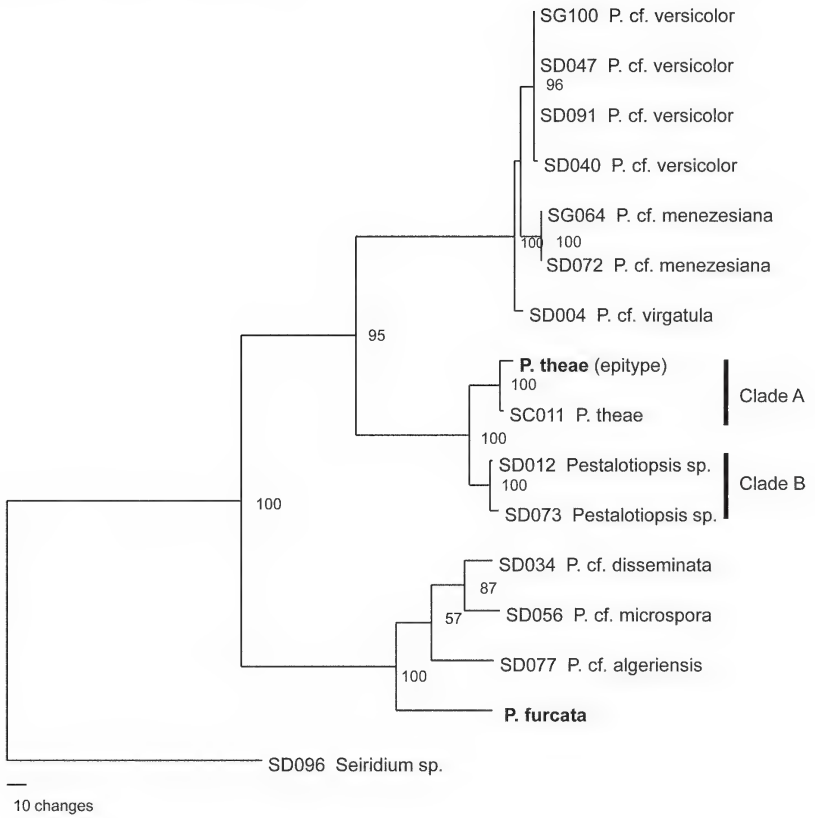

FIG. 5. Maximum parsimony phylogram generated from combine three genes (ITS, β-tubulin and TEF1) analysis of species of *Pestalotiopsis* recorded from tea. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* sp. is placed as outgroup, and all ex-type sequences are set in bold font.

isolates from tea plants at the Mushroom Research Centre with ITS sequences in GenBank and there was one similar species. The type strain of *P. hainanensis* is similar to the ITS sequence of the Thailand tea isolate, although morphological characters differentiate these species. In the phylogram (FIG. 5) the Thailand tea species forms a monophyletic subclade and is distinguished as the new species, *P. furcata*. Our analyses confirm the observations by Hu et. al. (2007) and Liu et al. (2010) that a combined multigene dataset better resolves the taxonomy of *Pestalotiopsis*. Hu et al. (2007) showed that the ITS gene is less informative than the β-tubulin gene in differentiating *Pestalotiopsis* species. Liu et al. (2010), however, disagreed and indicated that proper analysis and alignment of the ITS

region can usefully group *Pestalotiopsis* species according to different types of conidial pigmentation, which can be used as a key character for the phylogeny of the genus. We constructed phylogenetic trees separately for each gene region (results not shown for β-tubulin and TEF1) with ITS < β-tubulin < TEF1 in their degree of species resolution, but best resolution results were with all three genes combined.

Most DNA sequences deposited for *Pestalotiopsis* species in GenBank are problematic (Maharachchikumbura et al. 2011), as they are likely to have been wrongly named. There are very few ex-type sequences and therefore the resolution of species and their authentic naming are difficult and confused. Re-examination of type materials and establishment of epitypes with sequenced living cultures are needed to advance understanding of species in this genus.

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Critical revision of myxomycetes in the Buenos Aires BAFC herbarium — 1

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Abstract — The first analytical results of 32 myxomycetes specimens kept at the BAFC herbarium are presented. The following species stand out: *Craterium obovatum, Diderma meyerae, Diderma stellulum, Didymium ovoideum, Stemonitis pallida, S. webberi, Trichia decipiens* var. *olivacea*, and *T. subfusca*. Type material descriptions of *Stemonitis pallida* and *S. webberi* are also provided. Optical microscope (LM) and scanning electron microscope (SEM) micrographs illustrate the most representative characteristics of species that are rare or not often mentioned in the literature.

Key words — *Protozoa*, myxobiota, slime moulds, Argentina, USA, taxonomy

Introduction

The BAFC herbarium currently at the Biological Sciences Department of the School of Exact and Natural Sciences, Buenos Aires University, was created in the 1960s from Rolf Singer's 1960–66 collections and other materials. The herbarium later added donations from Irma Gamundí, Jorge E. Wright, Carlos Gómez, Emilio del Busto, and María E. Ranalli (among others), and Jorge W. Wright arranged for shipping mycological materials from the old Ministry of Agriculture and the Instituto Forestal Nacional to BAFC. During the 1970s, Jorge Deschamps, Alicia Godeas, Daniel Cabral, Silvia López, Mónica Adler, Andrea Romero and others continued with this expansion. During the last decade following the death of Jorge E. Wright, the fungal collections in the herbarium have not increased appreciably. The herbarium currently curates approximately 28,000 mycological specimens, including 476 myxomycetes.

In this paper, we present critical revisions of some rare or taxonomically significant myxomycetes kept in the BAFC herbarium. Some of this material originates from other countries.

Materials & methods

The material studied originated from Argentina (Buenos Aires, Santa Fé, Misiones, Tucumán, and Tierra del Fuego provinces), USA (New York, Maryland, and California states), and Brazil (Rio Grande do Sul state). The collections are preserved in the BAFC herbarium (School of Exact and Natural Sciences, University City, II Pavilion, 4th. floor, University of Buenos Aires, Argentina).

Slide mounts in Hoyer's medium of each specimen are preserved in the Plant Biology herbarium of the University of Alcalá, Madrid (AH, Alcalá de Henares). Spore measurements were made using an oil immersion objective and include such surface structures as warts and spines.

Scanning electron microscopy (SEM) micrographs were taken at the University of Alcalá de Henares using a Zeiss DSM-950 instrument. Sporocarps were rehydrated in concentrated ammonium hydroxide (28–30%) for 30 minutes, dehydrated in aqueous ethanol (70%) for 30 minutes, fixed for 2 hours in pure ethylene glycol dimethyl ether (= 1,2-dimethoxymethane) and finally immersed in pure acetone for at least 2 hours followed by critical point drying and sputtering with gold-palladium. This technique allows the use of very little material (part of a single sporocarp or even no more than a few spores).

The terminology used for the spore-producing stages follows Dörfelt & Marx (1990) and Lado & Pando (1997). Spore wall ornamentation as seen by SEM is described according to the terminology proposed by Rammeloo (1974, 1975) and abbreviations for author citations follow Kirk & Ansell (1992). The nomenclature used follows Lado (2001, 2005–11).

Taxonomy

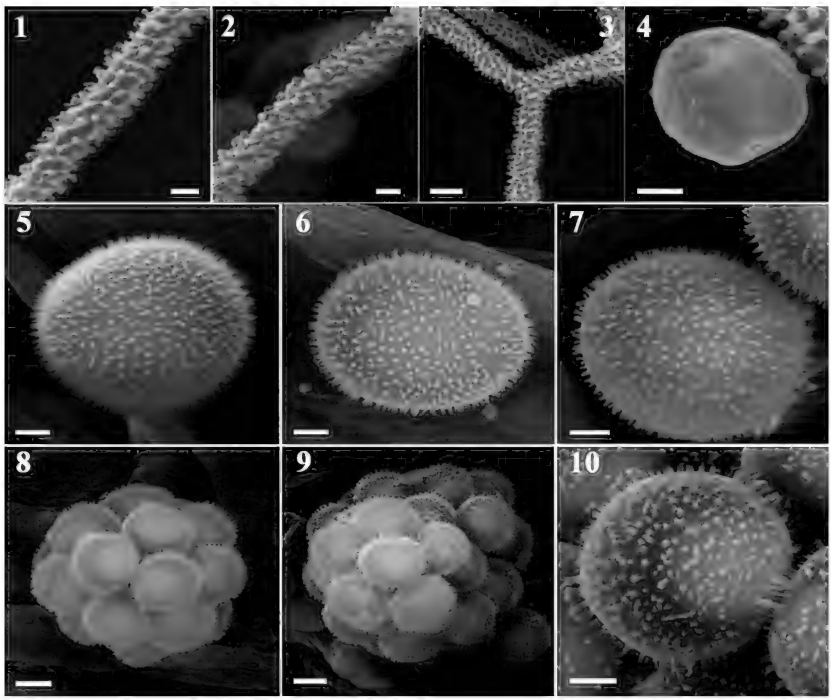
Arcyria globosa Schwein., Schriften Naturf. Ges. Leipzig 1: 64 (1822) FIGS. 1–4

SPECIMEN EXAMINED: **USA**: **NEW YORK**, Centreport, on crown debris of *Castanea* sp., VIII-1891, leg. O.F. Cook (BAFC 30398, ex "BPI 3405" [= BPI 805828]).

COMMENTS — This species is characterized by its isolated or gregarious, stipitate sporocarps (0.3–0.7 × 0.5–1.5 mm), globose, white to greyish white in colour, a fugacious peridium with a wide calyculus and concolorous stipe of 0.2–0.8 mm length. Capillitium elastic, formed by 3.5–4.5 µm diam. filaments with warts which are usually arranged in more or less marked spirals. Spores 7.5–8.5 µm in diam., hyaline, with dispersed warts. Under SEM, spore ornamentation is seen to comprise dispersed groups of irregular verrucae. The remaining spore wall contains a surface covered by tiny bacula, which cannot be seen under the light microscope (LM). The capillitial filaments contain warts that are joined in short crests with a helicoidal arrangement.

Badhamia affinis Rostaf., Sluzowce monogr.: 143 (1874) FIGS. 5–7

SPECIMEN EXAMINED: **ARGENTINA**: **BUENOS AIRES**, Banfield, on semi-rotten bark of *Manihot grahamii* Hook., 5-IV-1973, leg. J. Deschamps (BAFC 22822, as *Badhamia cinerascens*).



Figs. 1–10 ***Arcyria globosa*** BAFC 30398 (1–4): 1–3. Capillitium. 4. Spore (SEM). ***Badhamia affinis*** BAFC 22822 (5–7, SEM): Spores. ***Badhamia versicolor*** BAFC 22854 (8–10. SEM): 8, 9. Packets of spores. 10. Spore. Bars: 1, 2, 4–7, 10 = 2 µm; 3, 8, 9 = 5 µm.

Comments — This species is characterized by its globose to subglobose sporocarps which are laterally squashed (0.5–1 × 0.4–1 mm) and isolated or in groups of 2–5, sometimes forming short substipitate plasmodiocarps which are dark brown at the insertion with the substrate. The peridium is whitish and warted by the accumulation of calcium carbonate. Dehiscence is apical and irregular. The capillitium is a three-dimensional net of calcium carbonate tubules, which join in the centre of the sporotheca to form a whitish strongly marked pseudocolumella. Spores are 14–15(–16) µm in diam., globose to subglobose, dark violet, with abundant dense warts. Regularly distributed bacula form the spore ornamentation (SEM).

The presence of a marked white pseudocolumella at the centre of the sporotheca and the large spore size are two important diagnostic characters for *B. affinis*. See Moreno & Oltra (2010) for more information, including LM and SEM photographs, can be found in.

Badhamia cinerascens G.W. Martin, which is rarely cited, is a rare and critical species from Colombia. It differs from *B. affinis* by its lack of a pseudocolumella and thin peridium.

Badhamia versicolor Lister, J. Bot. 39: 81 (1901) FIGS. 8–10.

SPECIMEN EXAMINED: **ARGENTINA**, **TIERRA DEL FUEGO**, Puerto Harberton, on bark of possibly *Nothofagus pumilio* (Poepp. & Endl.) Krasser "lenga", 2-II-1973, leg. J.E. Wright & E. del Busto 2177 (BAFC 22854).

COMMENTS — This material was previously cited by Deschamps (1976). The specimen lacks calcium carbonate in the peridium, although it has some dispersed granules. Its clustered spores form thick hollow balls of 30–40 spores in each cluster. Individual spores are ovoid to triangular with obtuse extremes of 11–13 × 10–11 µm diam. and are more strongly warted on the outer exposed surface. Irregularly distributed bacula form the spore ornamentation (SEM).

This species is known only from Tierra del Fuego, Argentina (Arambarri 1975; Deschamps 1976) and Chubut in Patagonia (Wrigley de Basanta et al. 2010).

Craterium obovatum Peck, Bull. Buffalo Soc. Nat. Sci. 1: 64 (1873) FIGS. 11–13
= *Badhamia rubiginosa* (Rostaf.) Rostaf., Sluzowce monogr. suppl.: 5 (1876)

SPECIMEN EXAMINED: **USA**, **NEW YORK**, SUFFOLK CO., Centerport, VIII-1891, leg. O.F. Cook 1366, as *B. rubiginosa* (BAFC 28684).

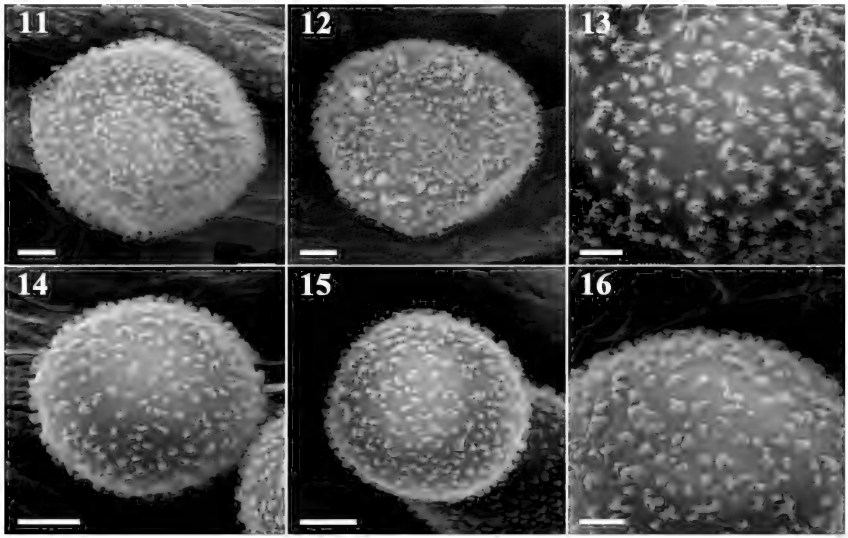
COMMENTS — This species is characterized by its stipitate sporocarps, which are isolated, gregarious to caespitose and form dense fructifications, 1–2 mm total height. Sporothecae are obovoid, 0.8–1 × 0.4–0.6 mm, brownish-purple and with a thin peridium. Dehiscence is apical and operculate. Stipe cylindrical, 0.5–1 mm in length and usually equal or shorter than the sporotheca. Pseudocolumella is not very well formed. The capillitium is formed by very abundant whitish nodules. Hypothallus dark brown, membranous. Spores are 12–15 µm in diam., globose to slightly polyhedric, dark violet, with tightly arranged warts that sometimes form crests. Dense bacula with warty coralloid apices form the spore ornamentation (SEM).

Excellent photographs of the sporocarps of this species can be found in Poulain et al. (2011). This typical and strange spore ornamentation (SEM) has been observed in specimens from Baja California and Sonora (Mexico) by Lizárraga et al. (1999, 2007).

Diderma meyerae H. Singer, G. Moreno, Illana & A. Sánchez, in Moreno et al., Cryptog. Mycol. 24: 53 (2003) FIGS. 14–16

SPECIMEN EXAMINED: **USA, CALIFORNIA**, SHASTA CO.,Sunflower Flat, Lassen Volcanic National Park, elevation 6000 feet, on conifer wood, 7-II-1965, leg. D.T. Kowalsky. (BAFC [ex Bridge Cooke Herbarium 34923] as *Diderma niveum*).

COMMENTS — The whitish to straw-colored fructifications are arranged in dense groups. Sporothecae are 1.5–3 mm diam., globose to subglobose, and sometimes compacted together. Dehiscence is apical and irregular. Peridium



Figs. 11–16. ***Craterium obovatum*** (BAFC 28684): 11, 12. Spores (SEM). 13. Spore ornamentation. ***Diderma meyerae*** (BAFC [ex Bridge Cooke Herbarium 34923]): 14, 15. Spores (SEM). 16. Spore ornamentation. Bars: 11–12, 14–15 = 2 µm; 13, 16 = 1 µm.

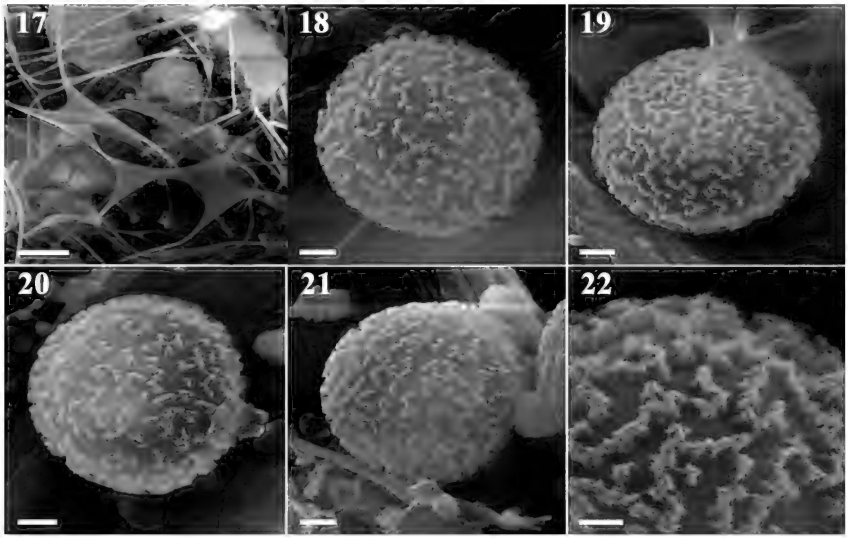
double, the external layer crustose and calcareous, and internal layer transparent to slightly iridescent. Pseudocolumella central, of variable morphology, globose to subglobose or conical with dark straw to ferruginous cream colours. Capillitium with dark filaments (1–2 µm width), lighter and bifurcate at the tips. Spores dark in mass, (7–)8–9 µm in diam., globose to subglobose, light violet (LM), with thick warts to short crests. Verrucae, some joining to form short crests, comprise the ornamentation (SEM).

Diderma meyerae is easily recognised under the LM from its scarcely projecting, non-spinose ornamentation formed by warts that join to form short crests. Moreno & al. (2003) described *D. meyerae* from European nivicolous material (Austria and France). This species is often confused with *D. niveum*. The European material has larger spores (10–13(–15) µm diam.), but the spore ornamentation and other characteristics are similar to those of the American specimen.

Diderma stellulum M.L. Farr, Int. J. Mycol. Lichenol. 3: 208 (1988) Figs. 17–22

Specimen examined: **BRAZIL, Rio Grande do Sul**, Porto Alegre, Morro Santana, 31-VIII-1984, leg. C. Rodriguez (BAFC 32313, as *D. stellula*).

Comments — Sporocarps isolated to gregarious, stipitate, dark brown, reminiscent of the colour of *Lepidoderma trevelyanii* (Grev.) Poulain & Mar.



FIGS. 17–22. ***Diderma stellulum*** (BAFC 32313: 17. Capillitium. 18–21. Spores (SEM). 22. Spore ornamentation. Bars: 17 = 10 μm; 18–21 = 2 μm; 22 = 1 μm.

Mey, 1.5–2 mm width when opened. Stipe approximately 1 mm height, 0.2 mm wide, dark brown and widened at its base. Peridium with three layers, the outer layer cartilaginous of a brown colour, the middle layer is whitish, thick with calcareous granules, the inner layer is attached to the middle layer which is membranous and iridescent. Dehiscence stellate, with recurved rays. Pseudocolumella not very prominent, 0.4–0.6 mm, flat, rough, whitish to straw-coloured. Hypothallus membranous. Capillitium, dark, reticulate, formed by thin filaments of 0.5–1 μm diam. and with wider nodules of 5–40 μm diam. Spores 12–14 μm diam., globose, dark brown and strongly warted. These warts are joined forming crests or a marked subreticulum; SEM shows that the spore ornamentation is formed by bacula joined in long sinuous crests, giving a somewhat subreticulate appearance.

Farr (1988), who described *D. stellulum* from a single specimen from Brazil, did not indicate where the species type is deposited. The BAFC specimen comes from the same type locality and was collected by the same person. Although the collection date is two years before the one indicated by Farr as "comm. 1986", it is possible that this specimen is part of the type, although Farr described a slightly larger spore (14–16 μm).

Diderma stellulum is macroscopically similar to *Lepidoderma trevelyanii,* which is differentiated by its peridial crystalline scales and smaller (11–12 μm) warted spores.

Diderma asteroides (Lister & G. Lister) G. Lister has different sporocarps and smaller (11–12 µm) warted spores.

Diderma antarcticum (Speg.) Sturgis differs in its sessile sporothecae, circumscissile dehiscence, globose columella, rugged, double peridium, capillitium filaments bearing small spines and spinose spores (Arambarri 1973; Wrigley de Basanta et al. 2010).

Diderma gracile Aramb. is a similar species which differs in its long stipe which is approximately twice the size of the sporotheca, irregular dehiscence forming petaloid non-recurved lobes, a pulvinate strongly marked columella and thin capillitial nodules (Arambarri 1973; Wrigley de Basanta et al. 2010).

Diderma subasteroides M.L. Farr, which is also stipitate, differs due to the irregular dehiscence, capillitium without marked nodules, and warted spores (Farr 1971). By SEM, the African specimens (Malawi and Rwanda) from Buyck (1983) have large warts that form a subreticulum similar to the specimen studied in this paper.

Didymium ovoideum Nann.-Bremek., Acta Bot. Neerl. 7: 780 (1958) FIGS. 23–26

SPECIMEN EXAMINED: **USA, MARYLAND**, Beltsville, on dead leaves, twigs, 7.VIII-1967, leg. M.L. Farr 4072 (BAFC 26535).

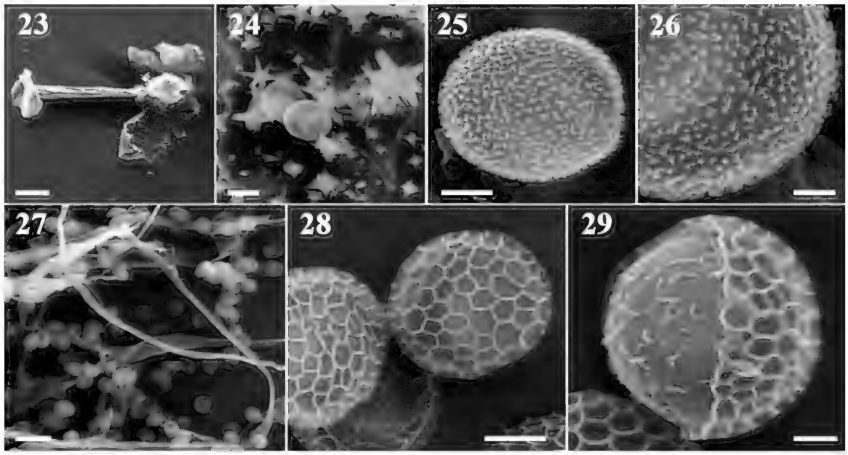
COMMENTS — This species is characterized by its large sporocarps, 1–1.2 mm total height and its stipitate and gregarious habit. Sporothecae 0.6–0.8 × 0.4–0.5 mm, subglobose to ovoid. Peridium thin, fragile with irregular dehiscence, whitish and pulverulent due to the accumulation of calcium carbonate crystals. Stipe approximately 1 mm long, cylindrical, reddish and longitudinally grooved. Pseudocolumella ovoid, yellow, covered with calcium carbonate crystals. The capillitium is branched and anastomosed and formed of hyaline to pale yellowish filaments approximately 1 µm diam. Spores 7–8.5 µm in diam., globose, dark to light violet, covered by small warts. Short, dense verrucae joined into low crests compose the spore ornamentation (SEM).

This species is known only in the Northern Hemisphere (www.discoverlife.org) and Central America (Costa Rica; Lado & Wrigley de Basanta 2008).

Physarum compressum Alb. & Schwein., Consp. fung. lusat.: 97 (1805)

SPECIMEN EXAMINED: **ARGENTINA, MISIONES**, El Pesado, on remains of *Bromeliaceae*, V-1964, leg. C.E. Gomez (BAFC 22130, as *P. polycephalum*).

COMMENTS — This species is characterized by its laterally compressed, whitish sporothecae with a black to whitish stipe, abundant short and rounded capillitial nodules, whitish in colour and globose spores of 10-12 µm diam. and warted ornamentation. In Argentina, according to (Deschamps 1976; Crespo & Lugo 2003), this species was recorded in the provinces of Buenos Aires, Jujuy, Misiones, and Tucumán.



Figs. 23–29. ***Didymium ovoideum.*** BAFC 26535 (24–26): 23. Sporocarp and columella (SEM). 24. Calcium carbonate crystals. 25. Spore (SEM). 26. Spore ornamentation. ***Reticularia jurana*** BAFC 22846 (27–29): 27. Pseudocapillitium. 28. Spore (SEM). 29. Spore ornamentation reticulate on one hemisphere and smooth on the other. Bars: 23 = 200 µm; 24 = 5 µm; 25, 28 = 2 µm; 26, 29 = 1 µm; 27 = 10 µm.

Physarum polycephalum Schwein., Schriften Naturf. Ges. Leipzig 1: 63 (1822)

Specimens examined: **ARGENTINA, Buenos Aires**, Tigre, 12-X-1968, leg. E. Barbetti (BAFC 22238); Belén de Escobar, El Cazador, on a rotting trunk of Salicaceae, 10-V-1969, leg. J. Deschamps & E. del Busto (BAFC 22232): As. Llavallol, Inst. Fit. Santa Catalina, on dry leaves and bark, III-1972, leg. A. Fortuny (BAFC 22610).

Comments — A widely distributed species, mostly found in the American continent (www.discoverlife.org). One cited collection has yellowish sporothecae, but two others are whitish as described in Martin & Alexopoulos (1969).

Reticularia jurana Meyl., Bull. Soc. Vaud. Sci. Nat. 44: 297 (1908) Figs. 27–29

Specimen examined: **ARGENTINA, Buenos Aires**, La Plata, Los Hornos, on living *Myoporum laetum* G. Forst., 6-I-1972, leg. J. Deschamps (BAFC 22846, as *R. intermedia*).

Comments — The studied specimen is fragmented but can be seen to develop from a dark reddish, pulvinate aethalium with a brilliant and very fragile peridium; abundant pseudocapillitium, delicate and non-rigid, formed by filaments that are flattened to form a three-dimensional net. Spores, dark in mass, 6–7 µm diam., reticulate on one hemisphere and smooth on the other. The SEM reveals that the spore ornamentation of one hemisphere is formed by a reticulum of 8–12 angular to hexagonal meshes with solid, unperforated muri; the other hemisphere is smooth or with a faint ornamentation.

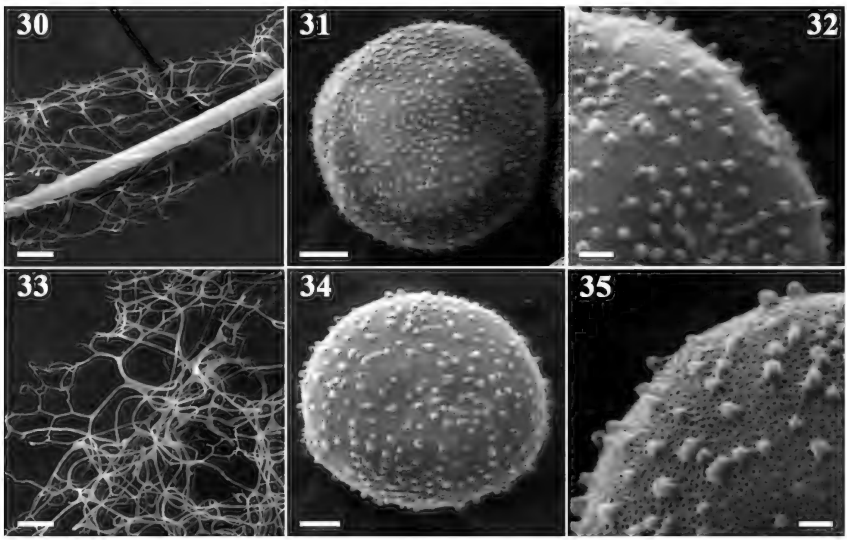
Reticularia jurana has been recently cited from Patagonia (Argentina) in *Nothofagus* woods (Wrigley de Basanta et al. 2010). In Argentina it has been confused with *Reticularia intermedia* Nann.-Bremek., which is distinguished by a pseudocapillitium formed by branched filaments that do not form a three-dimensional net.

Stemonitis axifera (Bull.) T. Macbr., N. Amer. Slime-moulds: 120 (1899) FIGS. 30–35
= *Stemonitis smithii* T. Macbr., Bull. Iowa Univ. Lab. Nat.Hist. 2: 381 (1893)

SPECIMENS EXAMINED: **ARGENTINA, BUENOS AIRES**, Llavallol, Santa Catalina, in logs, 2-III-1970, leg. L. Frias & G. Rovetta (BAFC 22306, as *S. flavogenita*); 2-III-1970, leg. J. Deschamps (BAFC 22310, as *S. flavogenita*).

COMMENTS — Sporocarps stipitate, 3.5-6 mm total height. Sporothecae dark to light reddish. Stipe cylindrical, dark reddish, 0.8-1.5(-2) mm length. Columella reaching to the apex. Capillitium with very open and wide meshes, more than 35 µm diam. Spores hyaline to slightly dark yellowish, (6-)7-8 µm diam., globose, almost smooth to lightly warted; (SEM) ornamentation of irregularly distributed verrucae to short bacula and a spore surface covered by a complete reticulum only visible in the SEM.

In the BAFC 22306 specimen, the columella apex has a small expansion resembling that of *Stemonitis flavogenita* but the spore characteristics confirm that it is *S. axifera*. Some collections of other *Stemonitis* species such as *S. fusca*



FIGS. 30–35. ***Stemonitis axifera***. BAFC 22310 (30–32): 30. Columella and capillitium. 31 Spore (SEM). 32. Spore ornamentation. BAFC 22306 (33–35): 33. Capillitium. 34 Spore (SEM). 35. Spore ornamentation. Bars: 30, 33 = 20 µm; 31, 34 = 2 µm; 32, 35 = 0.5 µm.

occasionally exhibit funnel-shaped expansions at the apex of the columella. The spore and capillitium characteristics need to be studied carefully to avoid misidentification.

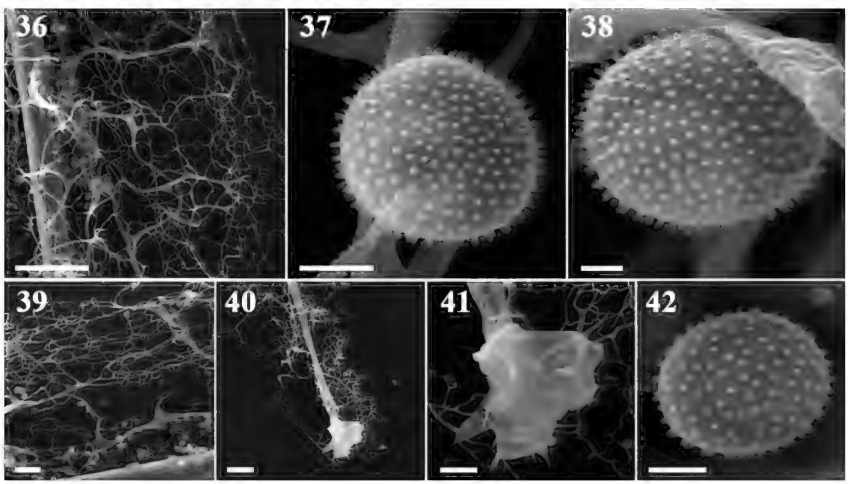
Stemonitis axifera is a cosmopolitan species (Martin & Alexopoulos 1969). Moreno & al. (2004) synonymized *Stemonitis smithii* with *S. axifera*, noting that although the spore dimensions varied, the spore ornamentation was the same.

Stemonitis flavogenita E. Jahn, Verh. Bot. Vereins Prov. Brandenburg 45: 165 (1904)
FIGS. 36–42

SPECIMENS EXAMINED: **ARGENTINA**, **BUENOS AIRES**, Castelar, INTA, I-1970, leg. E. del Busto & J. Deschamps (BAFC 22298): Llavallol, Santa Catalina, 6-IV-1970, leg. J. Deschamps (BAFC 22291).

COMMENTS — The specimens studied are characteristic for their stipitate aggregated sporocarps, 15–18(–20) mm total height. Sporothecae dark brown, cylindrical, 9–12 × 0.4–0.5 mm. Stalk cylindrical, dark reddish, 4–5 mm total height. Capillitium formed by filaments with short spines, forming an peridial net with meshes of 15–55 µm diam. and an internal net of very wide meshes. Columella widened into an apical funnel up to 120 µm diam. Spores globose, 7–8 µm diam., dark violet in mass, very light violet by LM, slightly warted. The spore ornamentation is formed by regularly distributed bacula (SEM).

Stemonitis flavogenita is very similar to *S. splendens* in spore ornamentation, but *S. splendens* lacks a columella funnel. This is a very widely distributed species, already recorded in Argentina (Deschamps 1976; Crespo & Lugo 2003).



FIGS. 36–42. ***Stemonitis flavogenita.*** BAFC 22291 (36–38): 36. Capillitium and columella. 37, 38. Spores (SEM). BAFC 22298 (39–42): 39. Capillitium. 40. Apex of columella. 41. Apex of columella (detail). 42. Spore (SEM). Bars: 36, 40 = 50 µm; 37, 42 = 2 µm; 38 = 1 µm; 39, 41 = 20 µm.

Stemonitis fusca Roth, Bot. Mag. (Römer & Usteri) 1(2): 26 (1787)

Specimen examined: **ARGENTINA**, **Buenos Aires**, Llavallol, Santa Catalina, Instituto Fitotécnico, 5-VI-1970, leg. J.E. Deschamps (BAFC 22296, as *S. virginiensis*).

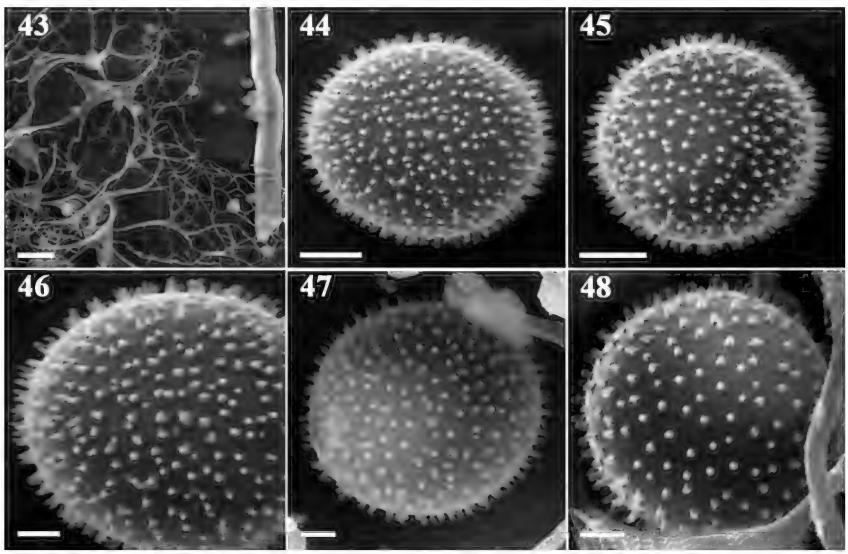
Comments — The spore ornamentation is reticulate with 8-10 meshes per hemisphere. This ornamentation and the larger spore size distinguishes *Stemonitis fusca* from *Stemonitis virginiensis* Rex. More information about these species can be found in Castillo et al. (1997).

Stemonitis pallida Wingate, in Macbride, N. Amer. Slime-moulds: 123 (1899)

Figs. 43–48

Specimens examined: **ARGENTINA**, **Buenos Aires**, Llavallol, Santa Catalina, leg. C. Naranjo, 26-VI-1964, (BAFC 22309); La Plata, Punta Lara, Arroyo Las Cañas, on log remains, 10-III-1971, leg. del Busto & J. Deschamps (BAFC 22289). **USA**: **Pennsylvania**, Delaware Co., on rotten wood, coll. Hugo Bilgram, Ellis & Everhart, North American Fungi Second series 3498 (BPI 802957, isotype).

Comments — This specimen is characterized by its aggregated and stipitate sporocarps of 4–6 mm total height. Stalk 1–2 mm long, less than half the height of the sporocarp. Sporotheca cylindrical, dark brown, 0.4–0.5 × 0.3–0.4 mm. Capillitium an internal net with wide expansions (10–15 µm) and large meshes. Peridial net irregular, formed by hyaline to very pale small meshes, 7–20 µm diam. with short spines which make it distinct from otherwise similar species.



Figs. 43–46 ***Stemonitis pallida.*** BAFC 22309 (43–46): 43. Capillitium (detail). 44, 45. Spores (SEM). 46. Spore ornamentation. BPI 802957 (Isotype): 47, 48. Spores (SEM). Bars: 43 = 20 µm; 44, 45 = 2 µm; 46–48 = 1 µm.

Spores globose, 7–8 µm diam., pale lilaceous by LM, with abundant and regularly distributed warts. The ornamentation is formed by dense, long, narrow bacula (SEM), similar to that of Wingate's type material from Pennsylvania (USA).

Stemonitis pallida is a species close to *S. splendens,* which produces taller and darker sporocarps, a uniformly coloured capillitium, and a larger mesh size in the peripheral net. *Stemonitis pallida* is a widely distributed species, already cited from Argentina (Deschamps 1972; Crespo & Lugo 2003).

Stemonitis splendens Rostaf., Sluzowce monogr.: 195 (1874)

Specimens examined: **ARGENTINA, Buenos Aires**, Lavallol, Santa Catalina, on rotten wood, 3-XI-1968, leg. J. Deschamps (BAFC 22196, as *S. webberi*); II-1970, leg. J. Deschamps (BAFC 22294. as *S. pallida*); possibly on bark of *Ulmus procera* Salisb., 2-III-1970, leg. J. Deschamps (BAFC 22290, as *S. carolinensis*); San Clemente del Tuyu, on fallen log possibly of *Populus* sp., 24-II-1971, leg. G. Pousadela (BAFC 22285, as *S. flavogenita*). **USA, Virginia**, Radnor Heights, on *Pinus virginiana*, 20-VI-1935, leg. C.L. Shear (BAFC 22562).

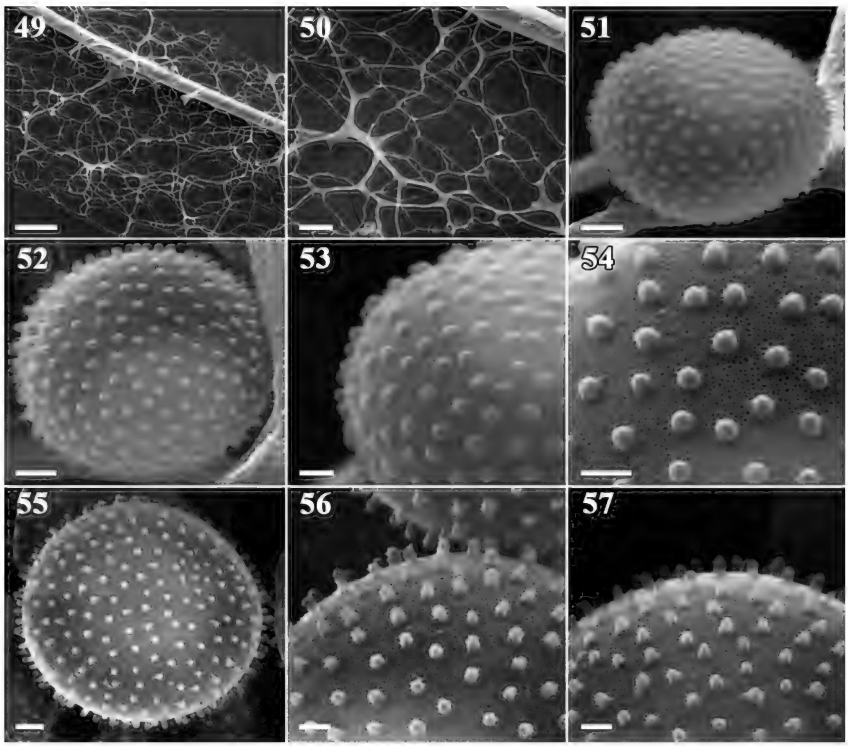
Comments — *Stemonitis splendens* is characterized by its grouped stipitate dark red sporocarps, 6–17(–20) mm total height. Sporothecae cylindrical, (8–)10–15 × 0.4–0.6 mm. Stalk dark red, cylindrical, hollow, 3–7 mm total height. Capillitium with branches more or less perpendicular to the columella, externally forming a net of wide meshes 50–80 µm diam. Spores globose, 6.5–8(–9) µm in diam., with regularly distributed warts. The SEM shows a spore ornamentation formed by abundant and evenly distributed bacula.

Stemonitis splendens is a species of variable size and morphology (Martin & Alexopoulos 1969). *Stemonitis flavogenita,* which displays a similar size and spore ornamentation under the SEM, is distinguished by a columellar apex lacking a membrane expanded into a cup-shape and smaller (15–55 µm) capillitial meshes. *Stemonitis pallida* can be differentiated by its shorter total height (4–6 mm) and a peridial net of hyaline to very pale meshes, less than 7–20 µm diam. and with short spines. SEM reveals spores with longer, denser bacula.

Stemonitis webberi Rex, Proc. Acad. Nat. Sci. Philadelphia 43: 390 (1891) Figs. 49–57

Specimens examined: **ARGENTINA: Buenos Aires**, Lavallol, Santa Catalina, on rotten wood, 9-V-1970, leg. L. Frias & G. Rovetta (BAFC 22297, as *S. carolinensis*); bark of *Ulmus procera*, 22-X-1972, leg. J. Deschamps & G. Rovetta (BAFC 22862, as *S. splendens*). **USA: Nebraska**, Lincoln, 1988, leg. H.J. Webber 15 (PH, type of *Stemonitis webberi*).

Comments — The Argentinean material is characterized by its sporocarps forming groups of 15–20 mm total height. Sporothecae cylindrical, dark brown, 9–12 mm high and 0.5–0.8 mm wide. Stalk dark red, 4–8 mm in length. Columella reaches to the apex where it gets thinner and becomes sinuous.



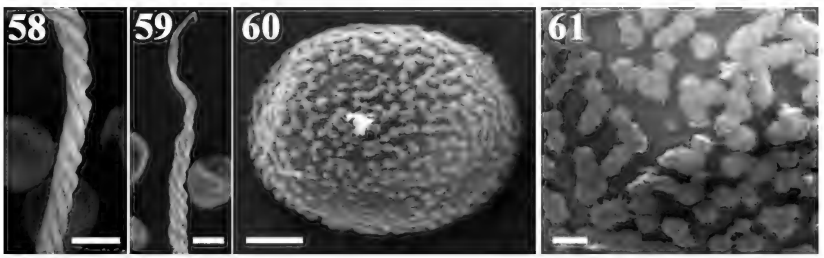
Figs. 49-57 ***Stemonitis webberi.*** BAFC 22297 (49–54): 49. Capillitium and columella. 50. Capillitium. 51, 52. Spores by SEM. 53, 54. Spore ornamentation. Type in PH: 55–57. 55. Spore (SEM). 56, 57. Spore ornamentation. Bars: 49 = 50 μm; 50 = 20 μm; 51, 55 = 2 μm; 52 = 1 μm; 53, 54, 56, 57 = 0.5 μm.

Capillitium is formed by an internal net of wide and angular meshes with an external net of wide meshes of more than 25 μm diam., formed by thin filaments, approximately 1–2 μm diam. and without spines. Spores globose to subglobose, (6.5–)7–8 μm in diam., dark violet, homogeneously warted. SEM reveals that the ornamentation is formed by regularly distributed thick bacula and that the spore surface is covered by a complete reticulum.

Stemonitis webberi type description: Sporocarps in groups; dark brown. Sporotheca cylindrical, 4–7 mm height. Stalk reddish of 3–4 mm height. Capillitium formed by an internal net of large wide meshes, membranous expansions in the anastomoses and a peripheral net slightly formed. Spores globose, 7–7.5 μm diam., light grey with a paler outline and evenly distributed spines. By SEM the spores have an ornamentation formed by regularly scattered bacula distributed over the entire spore surface, which appears to have a slightly reticulate ornamentation.

Stemonitis webberi, which shares with *S. splendens* a similar capillitium morphology and spore size, can be differentiated only by SEM due to its specific spore ornamentation.

Stemonitis axifera also has a similar reticulum on the spore surface that is observable only by SEM and which cannot be seen by LM. It can be distinguished by its capillitium with different meshes, different spore size, and an ornamentation with more scarce, irregularly distributed warts.



Figs. 58-61 ***Trichia subfusca*** (BAFC 22180): 58. Capillitial elater. 59. Free end of elater. 60. Spore (SEM). 61. Spore ornamentation. Bars: 58, 59 = 5 µm; 60 = 2 µm; 61 = 0.5 µm.

Trichia subfusca Rex, Proc. Acad. Nat. Sci. Philadelphia 42: 192 (1890) Figs. 58–61

Specimens examined: **ARGENTINA, Buenos Aires**, Punta Lara, Boca Cerrada, on rotten wood, 10-5-1969, leg. J. Deschamps (BAFC 22180, as *T. pusilla*). **USA, West Virginia,** Monongalia Co., Cooper's Rock State Park, Morgantown, 500 m altitude, on white-rotted logs of *Tsuga canadensis,* 16-IX-2000, leg. M. Schnittler & D.W. Mitchell (DWM 6171).

Comments — Sporocarps scattered and stipitate, 1.1–1.5 mm total height, stalk fibrous, darker than the sporotheca, 2–3 mm total height. Sporothecae globose to subpyriform, (0.4–0.5 mm diam.), dark brown with red shades. Peridium with apical irregular dehiscence, without dehiscence lines marked as in *Trichia botrytis* (J.F. Gmel.) Pers. Capillitium formed by yellow elaters of 3–4 µm diam., with three smooth spirals and free ends approximately 25 µm long. Spores pale yellow, 9–11 µm diam., regularly warted by LM. By SEM, the spores have relatively large capitate pila that tend to coalesce with adjacent pila (Rammeloo 1974).

Our material has a longer stalk than found in descriptions of *T. subfusca* by other authors as well as smaller spores (cf. 11–15 µm; Martin & Alexopoulos 1969). The American specimen of *T. subfusca* (DWM 6171), however, resembles our specimen in spore size and other dimensions.

Trichia decipiens var. ***olivacea*** (Meyl.) Meyl., Bull. Soc. Vaud. Sci. Nat. 55: 244 (1924)

Specimens examined: **ARGENTINA, Tierra del Fuego**, Valle Carbajal, in moss and hepatics on wood, 20-II-1961, leg. I. Gamundí (BAFC 22187, as *Trichia pusilla*); on the

road to Martial glacier, on rotten wood, 16-I-1964, leg. I. Gamundí (BAFC 22193, as *Trichia pusilla*).

Comments — This variety is characterized by its abruptly stipitate sporocarps, more or less operculate dehiscence, and spores with low crests.

Tubifera ferruginosa (Batsch) J.F. Gmel., Syst. nat. 2: 1472 (1792)

Specimen examined: **ARGENTINA**, **Entre Rios**, Departamento Victoria, Molino Doll, on bark of *Populus nigra* var. *italica* Koehne, 1-I-1972, leg. J. Deschamps (BAFC 22552, as *T. casparyi*).

Comments — This cosmopolitan species forms large pseudoaethalia with a well-developed hypothallus, usually over log debris. It is characterized by its reticulate spores (6–8 µm diam.) with uniformly sized meshes.

BAFC 22552 was misdetermined as *Tubifera casparyi* (Rostaf.) T. Macbr., a species with a central pseudocolumella, a structure absent from *T. ferruginosa* and BAFC 22552.

Tubifera microsperma (Berk. & M.A. Curtis) G.W. Martin, Mycologia 39(4): 461 (1947)

Specimens examined: **ARGENTINA**, **Tucumán**, Chicligasta, Alpachiri, 1-XII-1946, leg. A.Garolera (BAFC 30276 [ex LIL C-7550]). **Santa Fé**, Venado Tuerto, on fallen log, 23-III-1970, leg. D. Murray (BAFC 22253).

Comments — This species is easily recognized by its fructifications that usually form a stipitate pseudoaethalium, by small spores (4–5.5 µm diam.) and an inner verrucose peridium by LM and which, by SEM, appears as a surface with small raised tubules reminiscent of octopus suckers (Moreno et al. 2004).

Results

In this first contribution to the myxomycetes of BAFC, we concluded from our study of 32 specimens (26 from Argentina, 5 from the USA, 1 from Brazil) that:

(1) The 32 revised specimens represent 20 taxa (19 species and 1 variety) and resulted in redetermination of the following collections (Table 1):

(2) *Trichia decipiens* var. *olivacea* is added to the Argentinean myxomycete catalogue.

(3) *Diderma meyerae* is added to the USA and North American myxomycete catalogues.

(4) *Badhamia cinerascens*, *Reticularia intermedia*, *S. virginiensis*, and *Tubifera casparyi* are deleted from the Argentinean myxomycete catalogue.

(5) Type materials of *Stemonitis pallida* and *S. webberi* are revised and described.

Table 1. BAFC collections redetermined as a result of the current study

Previous incorrect label	Correct name
Badhamia cinerascens BAFC 22822	*Badhamia affinis*
Diderma niveum BAFC [ex Bridge Cooke Herbarium 34923]	*Diderma meyerae*
Physarum polycephalum BAFC 22130	*Physarum compressum*
Reticularia intermedia BAFC 22846	*Reticularia jurana*
Stemonitis carolinensis BAFC 22290	*Stemonitis splendens*
Stemonitis carolinensis BAFC 22297	*Stemonitis webberi*
Stemonitis flavogenita BAFC 22285	*Stemonitis splendens*
Stemonitis flavogenita BAFC 22306 and BAFC 22310	*Stemonitis axifera*
Stemonitis pallida BAFC 22294	*Stemonitis splendens*
Stemonitis splendens BAFC 22862	*Stemonitis webberi*
Stemonitis virginiensis BAFC 22296	*Stemonitis fusca*
Stemonitis webberi BAFC 22196	*Stemonitis splendens*
Trichia pusilla BAFC 22180	*Trichia subfusca*
Trichia pusilla BAFC 22187 and BAFC 22193	*Trichia decipiens* var. *olivacea*
Tubifera casparyi BAFC 22552	*Tubifera ferruginosa*

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New records of *Melampsora* species on willows in China

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Abstract — Three rust species, *Melampsora chelidonii-pierotii*, *M. humilis*, and *M. kiusiana*, are recorded for the first time in China based on morphological features and rDNA ITS1-5.8S-ITS2 sequence information. New hosts are also reported: *Salix raddeana* for *M. chelidonii-pierotii*; *S. caprea* and *S. siuzevii* for *M. humilis*; and *S. triandra* and *S. starkeana* for *M. kiusiana*. The morphology, host range, and distribution of these species in China are provided.
Key words — *Melampsoraceae*, *Salicaceae*, taxonomy

Introduction

The genus *Melampsora* Castagne (*Melampsoraceae*, *Pucciniales*) contains about 90 species (Kirk et al. 2008). The species are characterized by formation of crust-like telia comprising sessile, laterally adherent single-celled teliospores under the host epidermis (Pei et al. 2005). *Melampsora* species cause serious damage to cultivated willows, which are a significant resource for renewable biomass production (Royle et al. 1992). In China, 25 *Melampsora* species have been recorded on willows (Farr & Rossman 2011), of which 24 species were reported on ~72 cultivars representing 18 sections in *Salix*, while one species, *M. kamikotica*, was reported on *Chosenia* (Liu 2005, Tai 1979, Wang et al. 1980, Zhuang 1986, 1989, 1994, Zhuang & Wang 2006, Zhuang & Wei 2002, 2003). However, the taxonomic identities of these reported species need reevaluation due to taxonomic confusion and morphological similarity among species.

Melampsora species on willows deposited in several herbaria in China were investigated by morphological examinations and rDNA ITS1-5.8S-ITS2 sequence analyses. Three species — *M. chelidonii-pierotii*, *M. humilis*, and *M. kiusiana* — were represent new records for China. Their morphology, host plants, and geographical distribution in China are described in the present paper.

Materials & methods

Herbarium specimens used for morphological examinations were borrowed from the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences (HMAS); Inner Mongolia Agricultural University (HNMAP); the Mycological Herbarium of College of Forestry, Northwest A & F University, China (HMNWFC); Systematic Mycology and Microbiology Laboratory, Agricultural Research Service, USDA, USA (BPI); and the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan (TSH).

The collections were examined morphologically using light microscope (LM) and scanning electron microscopy (SEM). For LM examination, the urediniospores or thin sections of uredinia and telia were mounted in a drop of lactophenol solution on a microscopic slide. Germ pores of urediniospores were observed by the method of Liang (2006). For SEM, urediniospores or sori obtained from specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium with an E-1030 Ion Sputter Coater (Hitachi, Tokyo, Japan). They were examined using an S-4200 SEM (Hitachi, Tokyo, Japan) operating at 15 kV.

DNA was extracted from specimens by the methods of Tian et al. (2004) and fragments of rDNA ITS1-5.8S-ITS2 were amplified by polymerase chain reaction (PCR) techniques. Amplified PCR products were directly sequenced, and obtained sequences were compared with data deposited in GenBank using BLAST to calculate similarity values.

Taxonomy

Melampsora chelidonii-pierotii Tak. Matsumoto, Bot. Mag. (Tokyo) 40: 46, 1926

Fig. 1

Spermogonia and Aecia not seen in Chinese specimens. Uredinia mostly hypophyllous, 0.4–0.8 mm; Urediniospores obovoid, ellipsoid or broadly ellipsoid, 16–26 × 11–18 μm, wall 1.5–2.6 μm thick, echinulate, germ pores 3–6, bizonate or tending to be scattered; Paraphyses mostly pear- or lollipop-shaped and interspersed throughout uredinia, evenly thickened at apex or slightly thickened at apex, up to 7 μm. Telia amphigenous or epiphyllous, 0.2–0.4 mm, subcuticular; Teliospores 12–41 × 5–14 μm, wall 1.6 μm thick, not thickened at apex.

Specimens examined: **China**, **Shaanxi Province**, Shang Bai Yun, Tai Bai Shan, II, III on *Salix chaenomeloides* Kimura, 4 September 1985, Cheng-Ming Tian (HMNWFC13); **Jilin Province**, Antu county, II, III on *S. raddeana*

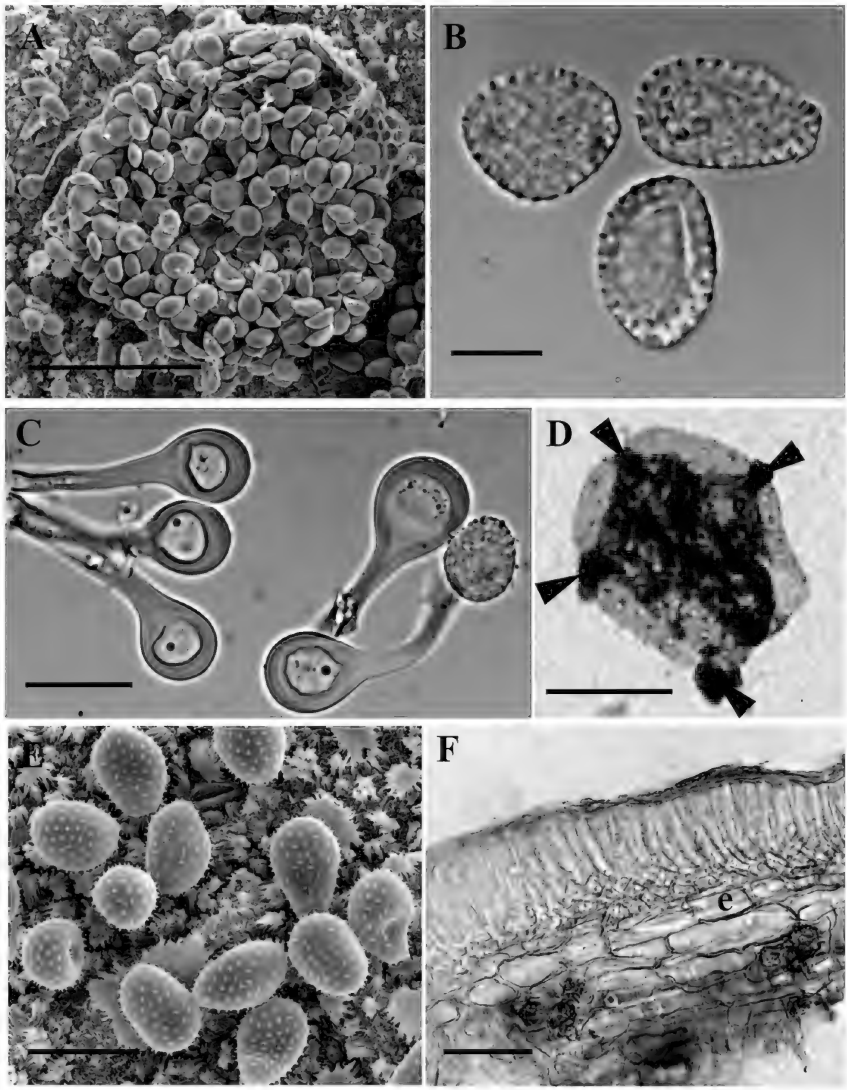

FIGURE 1. *Melampsora chelidonii-pierotii* (HMNWFC13). Morphology — A: Uredinium observed by SEM. B: Obovoid or ellipsoid urediniospores observed by LM. C: Paraphyses with evenly thickened apex or slightly thickened apex. D: Scattered germ pores (arrows). E: Echinulate urediniospores observed by SEM. F: Subcuticular telium (e = epidermal cells of the host). Scale bars: A = 100 μm; B, D = 20 μm; C = 10 μm; E = 15 μm.

Laksch. ex Nasarow, 16 July 1960, Yu-Chuan Yang, (BPI1109442); **ANHUI PROVINCE**, Qingyang County, II, III on *Salix* sp., 9 November 1932, Cheo S.Y. (BPI023209, BPI023210).

Comments: Matsumoto (1926) described *M. chelidonii-pierotii* based on a rust fungus on *S. pierotii* from Honshu, Japan. This species is characterized by subcuticular telia and mostly obovoid or ellipsoid urediniospores. Based on the morphology, one Chinese specimen on *S. chaenomeloides* from Shaanxi Province, which had previously been identified as *M. coleosporioides* (Cao & Li 1999), was redetermined to *M. chelidonii-pierotii,* as were three other specimens from China labeled as *M. larici-epitea* (one on *S. raddeana* and two on an unidentified *Salix* sp.). These specimens possessed subcuticular telia, and urediniospore dimensions agreed well with the descriptions of *M. chelidonii-pierotii* (Hiratsuka & Kaneko 1982). Two rDNA ITS1-5.8S-ITS2 sequences obtained from specimens BPI1109442 and BPI023210 also supported the morphological identification. A BLAST search of the GenBank database showed a 100% similarity of these sequences with *M. chelidonii-pierotii* strain E-1g (AB646769.1, deposited by Yamaoka et al. 2010). The teliospores of the Chinese specimens were smaller (12–41 × 5–14 µm) than in the Japanese specimens (18–60 × 14–23 µm, Hiratsuka & Kaneko 1982). However, the teliospore position separated these specimens from the morphologically similar species, *M. coleosporioides.*

Melampsora chelidonii-pierotii has been reported on *Salix pierotii* and *S. chaenomeloides* from Japan and Russia (Hiratsuka & Kaneko 1982; Azbukina 1974). Here we report *S. raddeana* as a new host. Although this rust species was identified on willows from Shaanxi, Jilin, and Anhui Provinces in China, a wide distribution has been expected throughout China based on the wide distribution of *S. chaenomeloides* in the northeast, northwest, and central regions the country (Zheng 1983). Japanese mycologists (Matsumoto 1926, Kaneko & Hiratsuka 1981) reported its aecial stage on *Chelidonium majus* var. *asiaticum* and *Corydalis incisa* and demonstrated experimentally the heteroecious life cycle between *S. chaenomeloides* and *Corydalis incisa.* However, host alternation of *M. chelidonii-pierotii* in China is still unknown.

Melampsora humilis Dietel, Bot. Jahrb. 32: 50, 1902 Fig. 2

Spermogonia and Aecia not seen in Chinese specimens. Uredinia mainly hypophyllous, 0.1–0.4 mm; Urediniospores globoid or broadly ellipsoid, 14–22 × 11–19 µm, wall 1.5–2.8 µm thick, echinulate, germ pores 3–7, scattered; Paraphyses mostly pear- or lollipop-shaped and peripheral or interspersed throughout uredinia, evenly thickened or slightly thickened at apex, up to 4.6 µm. Telia mainly hypophyllous, 0.1–0.4 mm, subepidermal; Teliospores 11–36 × 5–14 µm, wall sometimes slightly thickened at apex, 1.5–3.2 µm.

Specimens examined: **China, Hebei Province**, Xiao Wu Tai Shan, II, III on *S. caprea* L., 22 September 1990, Jian-Yun Zhuang (HMAS67392); **Inner Mongolia**, Genhe City, II, III on *S. siuzevii* Seemen, 19 August 1993, Zhen-Shi Hou (HNMAP1594. HNMAP3197).

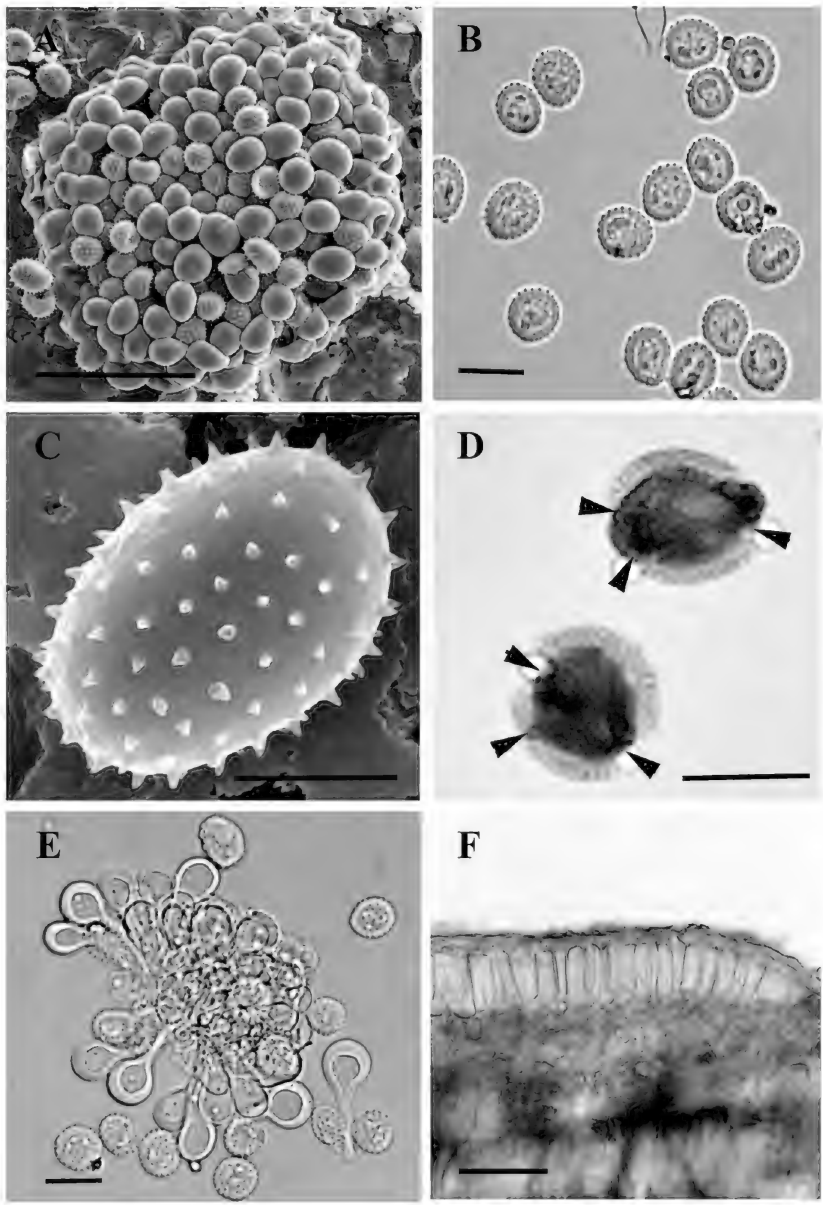

FIGURE 2. *Melampsora humilis* (HNMAP1594). Morphology — A: Uredinium with peripheral paraphyses. B: Globoid or broadly ellipsoid urediniospores observed by LM. C: Echinulate urediniospore observed by SEM. D: Scattered germ pores (arrows). E: Paraphyses slightly thickened at apex. F: Subepidermal telium with teliospores slightly thickened at apex. Scale bars: A = 50 μm; B = 20 μm; C = 6 μm; D, E = 15μm; F = 25 μm.

Comments: Dietel (1902) proposed *M. humilis* based on a rust fungus on *S. koriyanagi* in Japan. Morphologically, this species is closely related to *M. epitea* but differs from the latter by its shorter teliospores (Hiratsuka & Kaneko 1982). A Chinese rust specimen on *S. caprea*, collected from Hebei Province and previously identified as *M. larici-capraearum* (Tai 1979), was redetermined to *M. humilis*. Two rust specimens on *S. siuzevii* from Inner Mongolia, previously identified as *M. repentis* in China (Liu 2005), also represents *M. humilis*. Although the urediniospore dimensions and shape in these Chinese specimens agree with *M. epitea* as reported in Japan (Hiratsuka & Kaneko 1982), the teliospores are shorter (<36 µm) than in *M. epitea* (<50 µm). Sequence comparison also supported separation of the rust from *M. epitea*. The rDNA ITS1-5.8S-ITS2 sequences obtained from two specimens, HMAS67392 and HNMAP1594, showed a 99% similarity with sequences from *M. humilis* (TSH-R10561 and TSH-R10220) from *S. integra* in Japan (unpublished). The data were distinct from sequences of two formae specials of *M. epitea* (AY444777.2 and AY444778.2; 98% similarity) deposited by Pei et al. (2005).

Two willow hosts, *S. integra* and *S. koriyanagi*, were reported as hosts of *M. humilis* from Sakhalin, Taiwan, and Japan (Hiratsuka & Kaneko 1982). Here we report *M. humilis* for the first time for mainland China and *S. caprea* and *S. siuzevii* as new hosts. The aecial host and life cycle information of *M. humilis* have not been reported yet, and host alternation is also unknown for China.

Melampsora kiusiana Hirats. f., Bot. Mag. (Tokyo) 57: 281, 1943 Fig. 3

Spermogonia and Aecia not seen in Chinese specimens. Uredinia hypophyllous, 0.1–0.4 mm; Urediniospores globoid or broadly ellipsoid, 9–18 × 6–14 µm, wall 1.5–2.1 µm thick, echinulate, germ pore scattered or tending to bizonate, 2–5; Paraphysis pear- or lollipop-shaped and interspersed throughout uredinia, wall thickened at apex, up to 8 µm. Telia amphigenous, 0.1–0.4 mm, subepidermal; Teliospores 14–56 × 5–12 µm, wall 1 µm thick, slightly thickened at apex.

Specimens examined: **China, Inner Mongolia**, Gen He City, II, III on *S. triandra* L., 23 August 2005, Zhen-Shi Hou (HNMAP3161, HNMAP3181); Oroqen Qi, II, III on *S. starkeana* Willd., 23 August 1995, Zhen-Shi Hou (HNMAP1972).

Comments: Hiratsuka (1943), who described *M. kiusiana* on *S. subopposita* in Kyushu, Japan, characterized it with small urediniospores and slender teliospores. We redetermined two rust specimens on *S. triandra* from Inner Mongolia, previously reported as *M. repentis*, as *M. kiusiana*. A rust specimen on *S. starkeana*, identified as *M. lapponum* (Liu 2005), also resembled *M. kiusiana*. Morphological examinations revealed that the rusts specimens from *S. triandra* and *S. starkeana* had smaller urediniospores (9–18 × 6–14 µm) than other morphologically similar species, *M. epitea* (13–23 × 11–18 µm) and

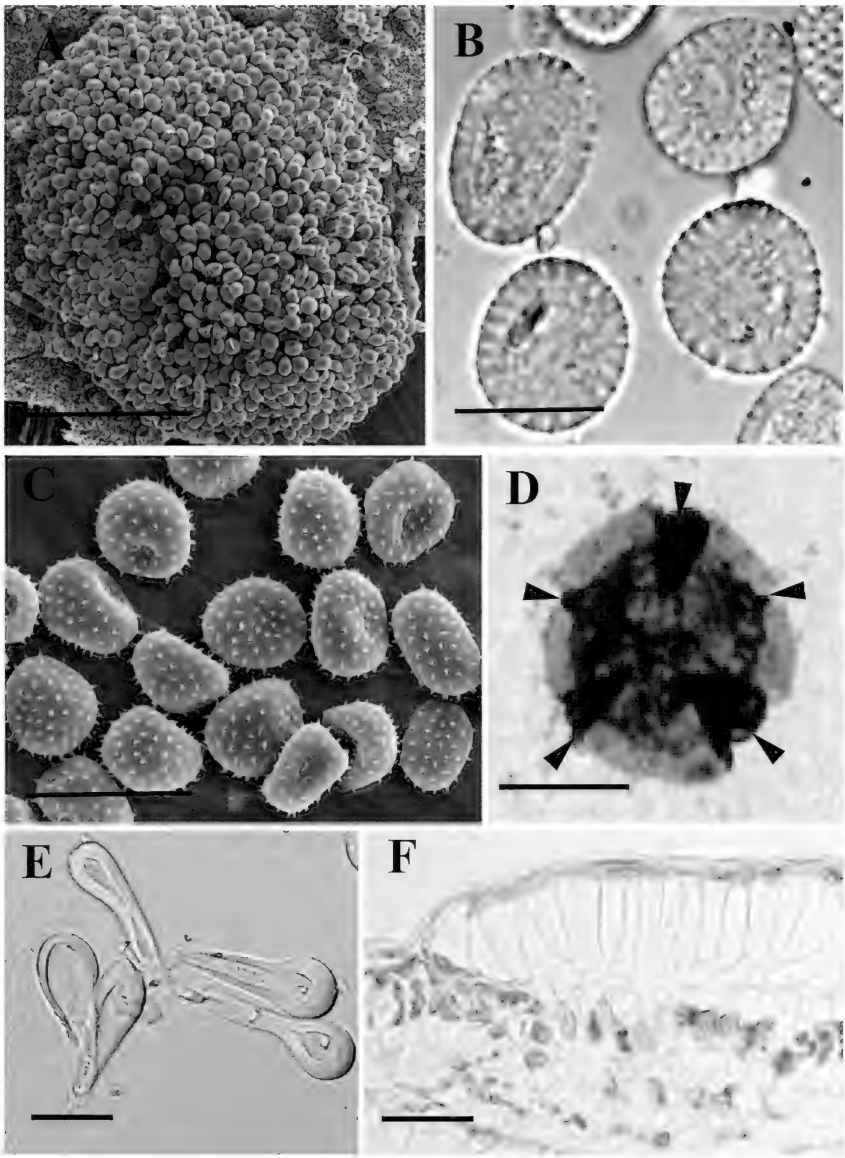

FIGURE 3. *Melampsora kiusiana* (HNMAP3181). Morphology — A: Uredinium with abundant paraphyses. B: Globoid or broadly ellipsoid urediniospores observed by LM. C: Echinulate urediniospores observed by SEM. D: Scattered germ pores (arrows). E: Paraphyses with evenly thickened or slightly thickened apex. F: Subepidermal telium with slender teliospores. Scale bars: A = 150 μm; B = 10 μm; C, E = 20 μm; D = 5 μm; F = 25 μm.

M. humilis (16–20 × 13–18 µm) (Hiratsuka & Kaneko 1982). The urediniospores of these specimens were also smaller than *M. repentis* (13–17 × 12–14 µm) and *M. lapponum* (20–21 × 15–16 µm) (Klebahn 1914). The teliospores were slightly larger than those of *M. epitea* (25–50 × 8–14 µm), *M. humilis* (13–33 × 6.5–15 µm), *M. repentis* (16–48 × 7–14 µm), and *M. lapponum* (30–50 × 6–12 µm) (Hiratsuka & Kaneko 1982, Klebahn 1914). Rusts on *S. triandra* and *S. starkeana* agreed with the morphological description of *M. kiusiana* (Hiratsuka & Kaneko 1982). Sequences from these specimens also differed from *M. epitea* and *M. humilis*. The rDNA ITS1-5.8S-ITS2 sequences obtained from HNMAP3161, HNMAP3181, and HNMAP1972 were somewhat distinct from *M. epitea* f. *laricis-epitea* (AY444778.2; 98% similarity) deposited by Pei et al. (2005). The sequences were also distinct from *M. humilis* (98% similarity) obtained from *S. caprea* and *S. siuzevii* in China. Morphological and molecular comparisons confirmed these Chinese specimens as *M. kiusiana*, and this represents the first report of *M. kiusiana* from Inner Mongolia in China. In Japan, *S. subopposita* was reported as a host of this species (Hiratsuka 1943), and the two willow species, *S. triandra* and *S. starkeana*, are new hosts of *M. kiusiana.* An aecial host of this species has not yet been reported for *M. kiusiana,* and life cycle information is also unknown for China.

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Eight new combinations and a replacement name in the genus *Hygrocybe*

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Abstract — Eight invalidly published combinations and one nomen novum in the genus *Hygrocybe* are validated in this paper.

Key words — *Hygrophorus*, valid publication

In Waxcap Mushrooms of Eastern North America (Bessette et al. 2012), eleven *Hygrophorus* names were invalidly transferred into the genus *Hygrocybe* because they lacked full and direct basionym references. Three of these eleven taxa (*H. murina*, *H. conica* var. *atrosanguinea* and *H. pratensis* var. *robusta*) had previously been validly combined by Malloch (2010). The remaining eight combinations are validated here. According to current interpretations of *Hygrocybe* by Bon (1976), Pegler (1983), Singer (1986), Young & Wood (1997), Young (2005), and Boertmann (2010), all these taxa should be transferred based on both their macroscopic characters and micromorphology.

It should, however, be noted that the genus *Hygrocybe* currently is under revision based on molecular information, and that significant changes in the taxonomy are expected (Lodge et al. 2006, Boertmann 2010).

Each of the taxa for which we propose a new combination has slender basidia, parallel to interwoven hymenophoral trama, and a pileipellis that is a cutis. Several of these taxa have been cited as *Hygrocybe* species in mushroom club newsletters and species lists as well as in the literature (e.g., Arora 1986, Barron 1999) without a valid combination (Boertmann 2002). In accordance with the rules of the International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012), we propose the following eight new combinations and one avowed substitute (nomen novum):

Hygrocybe americana Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **nom. nov.**
MycoBank MB801106
≡ *Hygrophorus acutus* A.H. Sm. & Hesler, Lloydia 5: 57. 1942.
non *Hygrocybe acuta* F.H. Møller 1945

Hygrocybe atro-olivacea (A.H. Sm. & Hesler) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801107
≡ *Hygrophorus atro-olivaceus* A.H. Sm. & Hesler, Sydowia 8: 326. 1954.

Hygrocybe auratocephala (Ellis) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801108
≡ *Agaricus auratocephalus* Ellis, Bulletin of the Torrey Botanical Club 6: 75. 1876.

Hygrocybe basidiosa (Peck) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801109
≡ *Clitocybe basidiosa* Peck, Bulletin of the New York State Museum of Natural History 1(2): 5. 1887.

Hygrocybe marginata var. ***concolor*** (A.H. Sm.) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801110
≡ *Hygrophorus marginatus* var. *concolor* A.H. Sm., Papers of the Michigan Academy of Science, Arts and Letters 38: 59. 1953.

Hygrocybe marginata var. ***olivacea*** (A.H. Sm. & Hesler) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801111
≡ *Hygrophorus marginatus* var. *olivaceus* A.H. Sm. & Hesler, Lloydia 5: 40. 1942.

Hygrocybe recurvata (Peck) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801112
≡ *Hygrophorus recurvatus* Peck, New York State Museum Bulletin 157: 28. 1912.

Hygrocybe subaustralis (A.H. Sm. & Hesler) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801113
≡ *Hygrophorus subaustralis* A.H. Sm. & Hesler, Lloydia 5: 46. 1942.

Hygrocybe tahquamenonensis (A.H. Sm. & Hesler) Bessette, A.R. Bessette, Roody & W.E. Sturgeon, **comb. nov.**
MycoBank MB801114
≡ *Hygrophorus tahquamenonensis* A.H. Sm. & Hesler, Sydowia 8: 331. 1954.

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We wish to thank David Boertmann for his recommendation to validate these names and for providing transfer data information. We also wish to thank both David Boertmann and Dr. Steve Trudell for reviewing this manuscript and offering valuable suggestions to improve it.

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Tuber subglobosum and *T. wenchuanense* — two new species with spino-reticulate ascospores

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Abstract — Two new *Tuber* species from China with spino-reticulate ascospores are described and illustrated. *Tuber subglobosum* is characterized by its subglobose ascospores with distinct spino-reticulate ornamentation, and *T. wenchuanense* is recognized by the very irregular spino-reticulate ornamentation on the ascospore surface, with the spines connected to each other at the base and forming U-shaped ridges at the apex. ITS sequence analyses support both new species.

Key words — *Ascomycota*, *Tuberaceae*, taxonomy

Introduction

Arguably the most useful feature for distinguishing *Tuber* species is the type of ascospore ornamentation. Some European species such as *T. aestivum* Vittad. and *T. magnatum* Picco have a more or less regular reticulum (mesh) covering the spore surface (Riousset et al. 2001). The other species group, which includes *T. melanosporum* Vittad. and *T. rufum* Vittad., has ascospores covered by spines. In contrast, many Chinese species have spino-reticulate ornamentation on the ascospore surface where a regular arrangement of spines is linked by U-shaped ridges. This was first described by Liu (1985) for *T. taiyuanense* B. Liu and again by Wang (1990) for *T. liaotongense* Y. Wang from Northeast China. More recently, other species with spino-reticulate ascospore ornamentation that have been described include *T. huidongense* Y. Wang, *T. microspermum* L. Fan & J.Z. Cao, *T. microspiculatum* L. Fan & Yu Li, *T. polyspermum* L. Fan & C.L. Hou, *T. sinoalbidum* L. Fan & J.Z. Cao, and *T. umbilicatum* Juan Chen & P.G. Liu (Chen et al. 2005, Fan et al. 2012a,b; Wang & He 2002).

Curiously, so far only one European species, *Tuber malacodermum* E. Fischer, and two North American species, *T. spinoreticulatum* Uecker & Burds. and

T. lyonii Butters, have been described with spino-reticulate ascospores (Chen et al. 2005). In this paper two additional species of *Tuber* with spino-reticulate ascospores are described from China.

Materials & methods

Morphological studies

The specimens of one *Tuber* species (collected in Sichuan Province) were already dried and deposited in HMAS (Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences). The specimens of the other new species were collected fresh from Sichuan and Yunnan Provinces and deposited in BJTC (Herbarium Biology Department, Capital Normal University). Macroscopic characteristics are described from both fresh and rehydrated specimens. Microscopic characters were described from razor-blade sections mounted in 3% KOH, Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed onto double-sided tape, mounted directly on an SEM stub, coated with gold-palladium, and then examined and photographed with a HITACHI S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLab E.Z.N.A. Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 µl reactions containing DNA template 2 µl, primer (10 µM) 2 µl each, 2× Master Mix

Table 1. *Tuber* specimens and sequence numbers used in molecular studies.

Species	Voucher specimen	ITS	ITS1	ITS2
T. aestivum	C. TaeW028I-E157 (UPS)	AJ888118	–	–
	C. TaeW064S-W140 (UPS)	AJ888062	–	–
T. huidongense	Thui-pzh08	DQ486032	–	–
	Thui-pzh09	DQ486031	–	–
T. liaotongense	IFS Y. Wang 88059	–	DQ478672	DQ478634
	IFS Y. Wang 88061	–	DQ478671	DQ478635
T. lyonii	GB 112	EU394704	–	–
	MOHARCN5Cfb	EU268568	–	–
T. rufum Picco	Truf-eu01	DQ329375	–	–
	R1	AF106892	–	–
	Morphotype 5	AY940646	–	–
	1506	AY112894	–	–
T. spinoreticulatum	RH158	GQ221454	–	–
	U188	FJ809884	–	–
T. subglobosum	BJTC FAN153	JX267043	–	–
T. taiyuanense	T42_HMAS 75888	GU979033	–	–
	HMAS 60234	–	DQ478664	DQ478650
T. wenchuanense	HMAS 60239	JX267044	–	–

(Tiangen Biotech (Beijing) Co. Ltd.) 25 µl. PCR reactions were run as follows: an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 2 min, 55 °C for 25 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). MP analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BP) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist & Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. *Tuber aestivum* was used as outgroup.

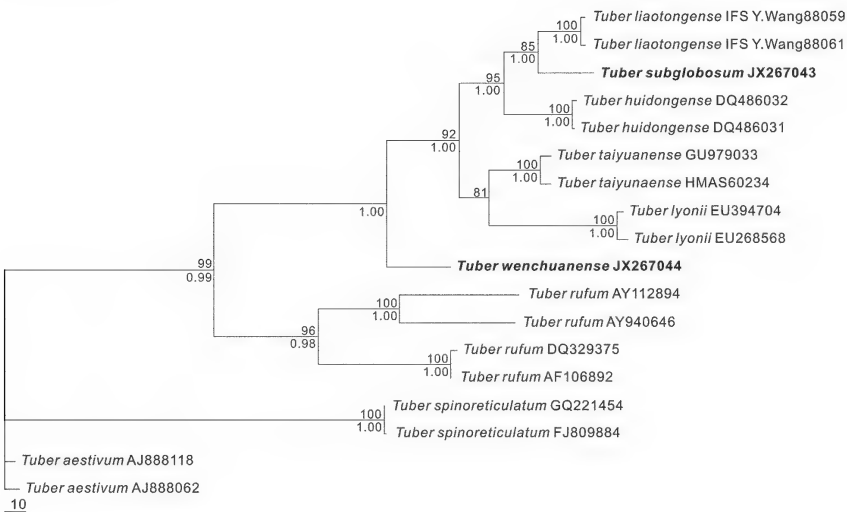

Fig. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of selected *Tuber* species with spino-reticulate ascospores, using *T. aestivum* as outgroup. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches. Bayesian posterior probabilities were estimated and clades with PP>0.95 (95%) are marked under the branches.

Results

Molecular phylogenetics

The maximum parsimony analysis of ITS sequences produced one most parsimonious tree (Fig. 1) with a length (TL) = 708 steps, consistency index (CI) = 0.7345, retention index (RI) = 0.7968, homoplasy index (HI) = 0.2655, and rescaled consistency index (RC) = 0.5852.

The ITS sequence analysis (Fig. 1) revealed that the sequence of the new species *T. subglobosum* grouped in a clade with the *T. liaotongense* sequences with moderate support (BP = 85, PP = 1.00). The *T. wenchuanense* sequence was placed as a separate clade in *Tuber*.

Taxonomy

Tuber subglobosum L. Fan & C.L. Hou, **sp. nov.** Fig. 2

MycoBank MB 800576

Differs from other *Tuber* species by the combination of subglobose ascospores and spino-reticulate spore ornamentation.

Type: China. Sichuan Province, Huili County, in the soil under mixed woods, 20 Dec. 2010, Jin-Zhong Cao116 (**Holotype**, BJTC FAN153).

Etymology: *subglobosum* (Lat.), referring to the subglobose ascospores.

Ascomata subglobose, 1–2 cm in diam., firm, solid, surface very minutely flattened verrucose, glabrous, brown at maturity. Odor slight, not pungent. Peridium 200–300 μm thick, two layers; outer layer 100–150 μm, pseudoparenchymatous, composed of small subangular or subglobose cells mostly 7.5–15 μm in diam., with thickened and yellowish-brown walls, darker towards the outer surface; inner layer 100–150 μm, composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 μm in diam. Gleba grey brown to brown at maturity, marbled with numerous, narrow, branched, white to white-yellow veins continuous with inner peridium. Asci subglobose, ellipsoid, clavate, or irregular, hyaline, thick-walled, mostly 2–4-spored, rarely 1-spored, with a short or a very long stalk, 75–100 × 45–70 μm excluding stalk. Ascospores mostly subglobose, a few broadly ellipsoid, dark brown at maturity, 20–32.5 × 20–27.5 μm excluding ornamentation; ornamentation distinctly spino-reticulate, with spines 3–6 μm high, straight or bent at apex, the meshes generally 4–6 across the ascospore width.

Additional specimen examined: **China. Yunnan Province**, Huize County, in the soil under mixed woodland, 2 Nov. 2011, Shao-Ping Li 007 (BJTC FAN168).

Comments — *Tuber subglobosum* is very similar to *T. liaotongense*, an endemic species from Northern China. *Tuber liaotongense* also has subglobose to broad elliptic ascospores with spino-reticulate ornamentations, but its spines are much lower on the reticulum, making the ornamentation look like a typical reticulum (Cao et al. 2011). This alone is sufficient to distinguish the new

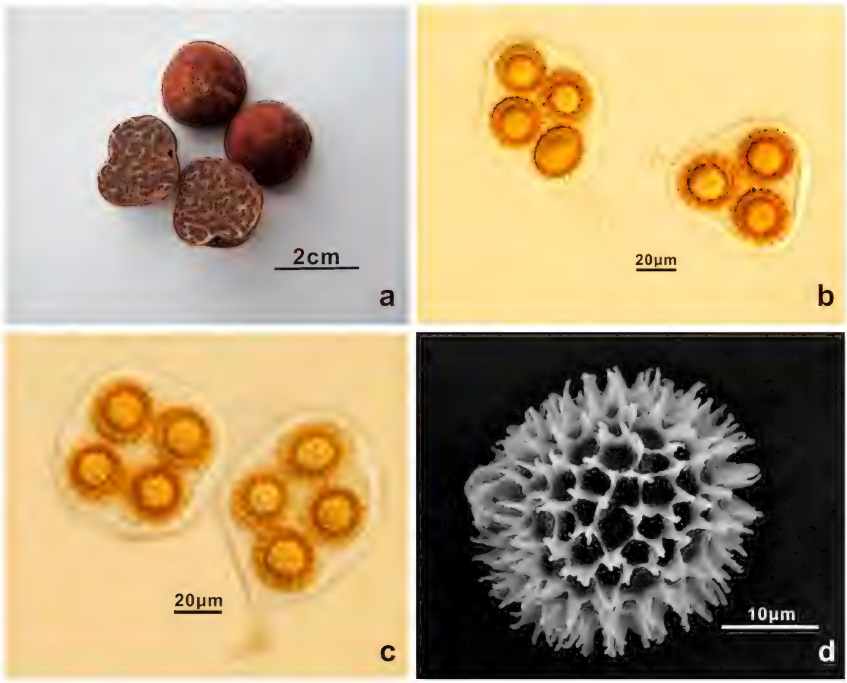

Fig.2. *Tuber subglobosum* (BJTC FAN153, holotype) a. Ascomata. b, c. Asci and ascospores observed under light microscope. d. Ascospore observed under SEM.

species. The phylogenetic analysis (Fig. 1) also grouped *T. subglobosum* in a clade with *T. liaotongense* but with only moderate support (BS = 85, PP = 1.00). This indicates that the two species are closely related but clearly distinct.

Other *Tuber* species with spino-reticulate ascospores differ from *T. subglobosum* by their more or less regular ellipsoid ascospores.

Tuber wenchuanense L. Fan & J.Z. Cao **sp. nov.** Fig.3

MycoBank MB 800577

Differs from the other *Tuber* species by its complete, deep and irregular spino-reticulate ascospore ornamentation with the spines connected to each other at both the bases and the tips with U-shaped ridges.

Type: China. Sichuan Province, Wenchuan County, in soil under *Larix mastersiana* Rehder & E.H. Wilson, 22 Sept. 1989, Bin-Cheng Zhang 618 (HMAS 60239, **Holotype**); Bin-Cheng Zhang 622 (**Isotype,** HMAS 60241A).

Etymology: *wenchuanense* (Lat.), referring to the type locality of the taxon.

Ascomata globose or subglobose, 0.5–1 cm in diam., grey brown when fresh, slightly pitted and whitish at the base, surface smooth. Odor not know

Fig.3. *Tuber wenchuanense* (HMAS 60239, holotype) a. Ascomata. b. Asci and ascospores observed under light microscope. c, d. Ascospore observed under light microscope. e. Ascospore observed under SEM.

when fresh. Peridium 200–250 μm thick, two layers; outer layer 50–100 μm, pseudoparenchymatous, composed of subglobose or subangular cells 5–15 μm in diam., with slightly thickened walls, yellowish-brown towards the surface; inner layer 150–200 μm, composed of intricately interwoven hyphae, hyaline, thin-walled, branched, septate, 2.5–5 μm diam. Gleba white, pale white or grey brown at maturity, marbled with numerous, narrow, branched, white veins continuous with inner peridium. Asci subglobose, ellipsoid or irregular, hyaline, thin-walled, 65–95 × 50–70 μm, sessile, 1–5-spored. Ascospores ellipsoid or broadly ellipsoid, yellow brown, covered with spines at first, then adjacent spines being joined by U-shaped ridges forming a complete irregular spino-reticulum, 25–45 × 17.5–30 μm excluding the ornamentation of 3–4 μm in height, occasionally on some ascospores the tips of spines remain isolated when the spines reaching up to 5–7.5 μm in height and reflexed at the tips, meshes numerous and various, typically 8–14 across the ascospore width.

Comments — The type specimen of *T. wenchuanense* in HMAS had been labeled as *T. lyonii*, a species originally from North America which, according to Trappe et al. (1996), is a species with ascospores with isolated spines or spines with bases partially connected by low ridges that form a complete or incomplete reticulum. However, our molecular analysis showed that the Chinese material

did not group in the same clade as *T. lyonii*, and instead belonged to a clade of its own (Fig. 1). Because of this and the unique ornamentation of its ascospores, we treat the Chinese material as a new species.

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Cordochaete (Agaricomycetes), a new corticioid genus from India

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Abstract – A new monotypic corticioid genus, *Cordochaete*, is described with the new species *C. cystidiata*, from Haridwar in Uttarakhand, India.

Key words – hyphal cordons, Chandighat, angiospermous wood

While conducting a fungal foray in Chandighat area in district Haridwar of Uttarakhand, India, S.K. Sanyal collected a specimen on decaying angiospermous wood in a mixed forest. The specimen was compared macroscopically and microscopically with similar genera in *Corticiaceae* (Rattan 1977, Eriksson et al. 1981, Burdsall 1985, Dhingra 1987, Nakasone & Sytsma 1993, Greslebin et al. 2004, Martínez &Nakasone 2005, Zmitrovich & Ezhov 2011) but could not be assigned to any already known, and is described here as a new genus. Morphological traits show similarities with the genera *Phlebiopsis* and *Rhizochaete*.

Cordochaete Sanyal, Samita, Dhingra & Avneet P. Singh, **gen. nov.**

MycoBank 801039

Differs from *Phlebiopsis* in having hyphal cordons and is distinguished from *Rhizochaete* by the absence of a red or violet KOH reaction in both the hymenium and hyphal cordons.

Type species: *Cordochaete cystidiata* Sanyal et al.

Etymology: The generic name is based on the resemblance to *Rhizochaete* and the presence of hyphal cordons.

Basidiocarp resupinate, adnate, effused; hymenial surface corneous as dry; hyphal system monomitic; generative hyphae without clamps; basal zone very thin, context conspicuous, of loosely interwoven hyphae; subhymenium compact, hyphal cordons both in context and subhymenium; cystidia thick-walled, encrusted; basidia clavate, 4-sterigmate, without basal clamp; basidiospores ellipsoidal to subcylindrical, inamyloid, acyanophilous.

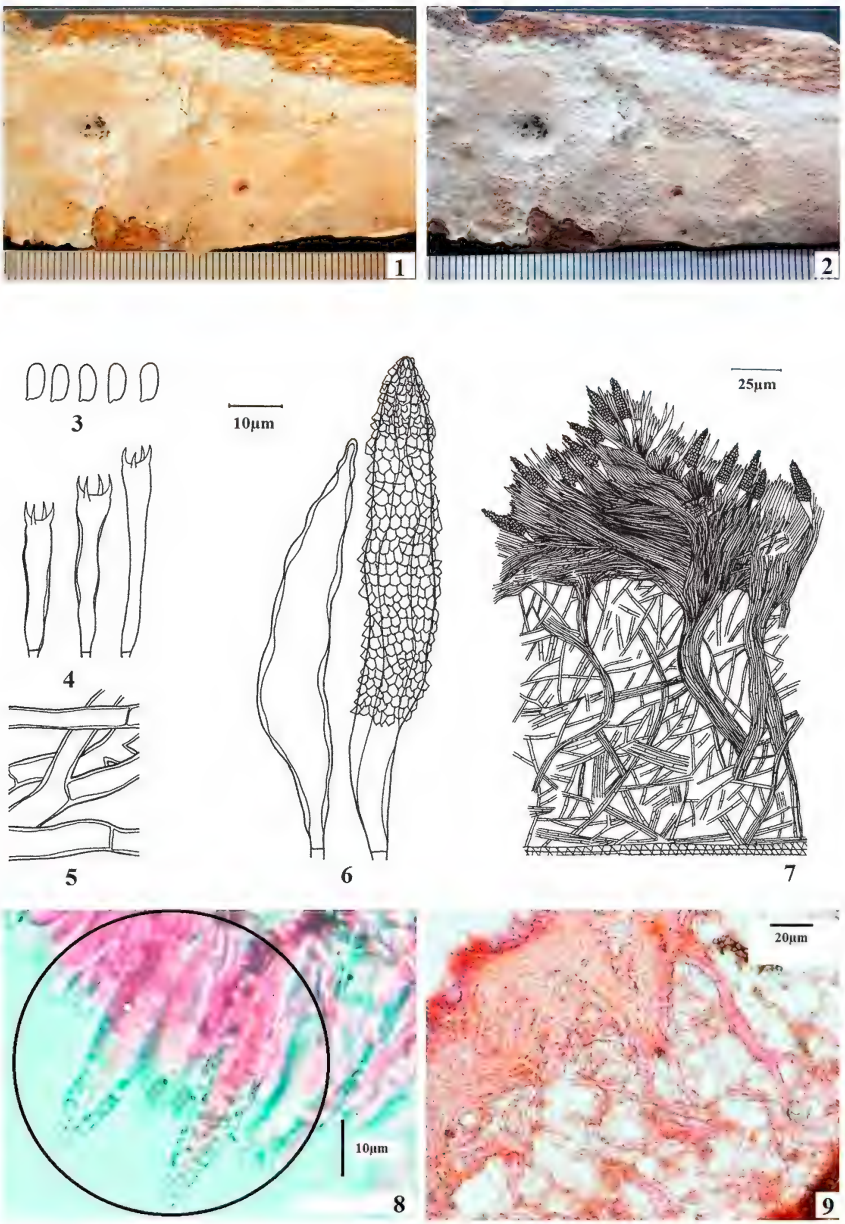

Plate 1. *Cordochaete cystidiata* (holotype). Figs 1–2: Basidiocarps. 1. Fresh. 2. Dried. Figs 3–7: Microscopic structures. 3. Basidiospores. 4. Basidia. 5. Generative hyphae. 6. Cystidia. 7. Section. Figs 8–9. Micrographs. 8. Encrusted cystidia and basidiospores. 9. Section.

Remarks— This genus can be differentiated from *Rhizochaete* in lacking a reddish or purplish change of the basidiome and hyphal cordons in KOH solution and from *Phlebiopsis* in lacking very compact context.

Cordochaete cystidiata Sanyal, Samita, Dhingra & Avneet P. Singh, **sp. nov.**

MycoBank 801040 Figs 1–9

Differs from *Phlebiopsis gigantea* by its loosely arranged context and presence of cystidia only in the hymenium and from *Rhizochaete radicata* by its compact hymenium and subhymenium.

Type: India, Uttarakhand: Haridwar, Chandighat, on decaying angiospermous wood, 20 August 2011, S.K. Sanyal 4745 (PUN, **holotype**).

Etymology: The epithet refers to the presence of encrusted cystidia.

Basidiocarp resupinate, adnate, effused, ≤ 850 µm thick in section; hymenial surface reticulate, corneous as dry, pale orange to grayish orange when fresh, orange gray to brownish gray on drying; margins thinning, fibrillose, paler concolorous to whitish, to indeterminate. Hyphal system monomitic; generative hyphae ≤ 6.5 µm wide; generative hyphae branched, septate, without clamps; basal zone of a very few horizontal hyphae, followed by conspicuous zone of loosely interwoven hyphae interspersed with hyphal cordons; subhymenial zone mainly of compact vertical hyphae and hyphal cordons; hyphae in the cordons thick-walled, without clamps. Cystidia 85–95 × 11–13.5 µm, subfusiform to fusiform, thick-walled, encrusted, projecting beyond the hymenial surface, negative to sulphovanillin. Basidia 28–39 × 5–6.5 µm, clavate, 4-sterigmate, thin- to somewhat thick-walled, without basal clamp; sterigmata ≤ 5 µm long. Basidiospores 6.5–8 × 3–3.5 µm, ellipsoidal to subcylindrical, thin- to somewhat thick-walled, inamyloid, acyanophilous.

Remarks— *Rhizochaete radicata* differs in having basidiome that turns reddish purple in KOH solution and subicular hyphae with brown granules that dissolve in KOH. *Phlebiopsis gigantea* differs in having cystidia distributed throughout the basidiome.

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New or rare fungi from eastern Amazonia. 1. *Circinoconiopsis amazonica* gen. and sp. nov.

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Abstract — *Circinoconiopsis amazonica*, a new fungal genus and species, is described and illustrated from specimens collected during an excursion to the Scientific Station Ferreira Penna at the Floresta Nacional de Caxiuanã, eastern Amazonia, Pará, Brazil.

Key words — helicosporous microfungi, palm tree, systematics, tropical rainforest

Introduction

The helicosporous anamorphic fungi include a rather small group of fungi recognized for their peculiar coiled conidia. These may be coiled in one, two, or three planes. In some genera such as *Candelabrum* Beverw., *Helicofilia* Matsush., and *Spirosphaera* Beverw., bizarrely shaped conidia form after a very complicated coiling and branching process.

From the morphological and ecological point of view, the helicosporous microfungi are fascinating fungi that are mostly saprobes associated with plant litter, rotten wood, and decaying twigs in moist places or around water (Zhao et al. 2007).

In an impressive paper on helicosporous microfungi from China, Zhao et al. (2007) published a checklist including the fifty-four known genera of this group of fungi. Intensive studies of mycobiota, mainly in tropical regions, have led to the discovery of new taxa among these captivating fungi.

The dematiaceous anamorphic fungus that is the subject of this paper was collected on decaying material of *Oenocarpus* sp., a kind of palm tree. Its conidia closely resemble those of the monotypic genus *Circinoconis* Boedijn. Boedijn erected *Circinoconis* in 1942 based on specimens associated with *Flagellaria indica* L. (*Flagellariaceae*) in Sumatra, *Imperata arundinacea* Cirillo (*Poaceae*) in Malaysia, and *Plectocomia elongata* Mart. ex Blume (*Arecaceae*) in Java. For

many years no new *Circinoconis* records were published in any part of the world. During palynological studies of peat samples from Tripura, India, Prasad & Ramesh (1984) found dispersed conidia, which were radiocarbon dated at 3340 ± 140 Y.B.P. Comparing the fossil conidia with those of *C. paradoxa*, the authors concluded that "... spores dealt presently are not known to be borne by any other fungus, except *Circinoconis*." *Circinoconis* was found again, 67 years after the first record, on *Calamus* (*Arecaceae*) in Thailand by Pinnoi et al. (2009). The present fungus is sufficiently different to be proposed here as a new genus, *Circinoconiopsis*.

Materials & methods

During an excursion in October 2003 to the Scientific Station Ferreira Pena at the Floresta Nacional de Caxiuanã, eastern Amazonia, Pará, Brazil, (1°42.5′–2°15′S 51°15′–51°56′W) samples of decaying parts of palm trees were collected. Conidiophores and conidia were extracted from the substratum using a fine sharpen needle under the dissecting microscope and mounted in lactic acid in glycerol (Kirk et al. 2008). Microscopic observations were made under clear light compound microscope. Herbarium acronyms follow the Index Herbariorum (Holmgren & Holmgren 2012).

Taxonomy

Circinoconiopsis A. Hern.-Gut., **gen. nov.**

IndexFungorum IF 550073

Differs from *Circinoconis* in lacking trichotomous apical branching of conidiophores and in having conidiogenous cells that produce only one conidium each.

Type species: *Circinoconiopsis amazonica* A. Hern.-Gut.

Etymology: Latin, *circinoconi-* referring to the hyphomycete genus *Circinoconis* = resemblance; Greek, -opsis, (ὄψις).

Anamorphic. Conidiophores macronematous, mononematous, erect, rarely branched. Conidiogenous cells monoblastic, integrated, determinate. Conidial secession schizolytic. Conidia solitary, coiled in one or two planes, rostrate, dry.

Circinoconiopsis amazonica A. Hern.-Gut. **sp. nov.** Fig. 1

IndexFungorum IF 550072

Differs from *Circinoconis paradoxa* in lacking trichotomous apical branching of conidiophores, and in having conidiogenous cells that produce only one conidium each.

Type: Brazil, Pará, Melgaço, Caxiuanã, Scientific Station Ferreira Penna, on decaying leaves of *Oenocarpus* sp. (*Arecaceae*), 2. X. 2003, A. Hernández. (**Holotype,** MG-203121).

Etymology: the specific epithet refers to the Amazon forest, the place of collection.

Anamorphic fungus. Conidiophores in natural substratum effuse, hairy, originated from mycelium immersed in the substratum; macronematous,

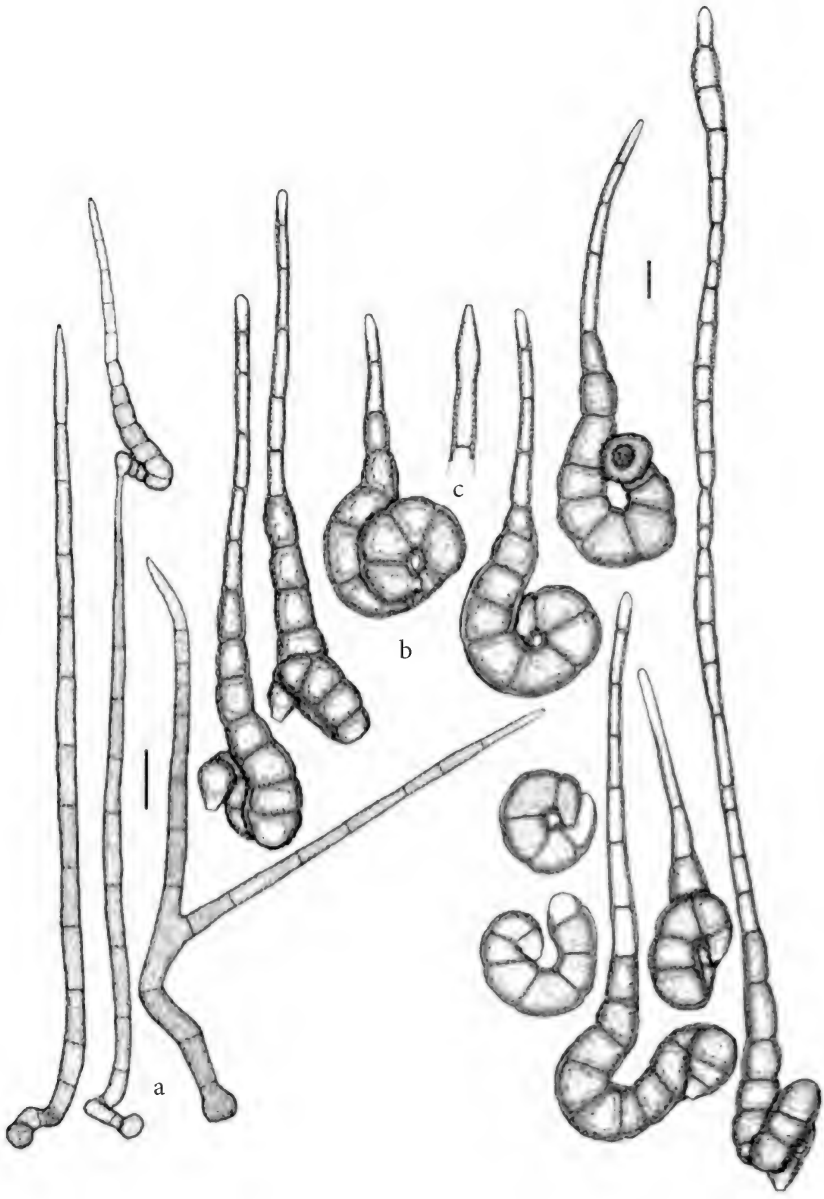

Fig. 1. *Circinoconiopsis amazonica* (holotype, MG-203121).
a: Conidiophores and conidium; b: Conidia; c: Detail of conidiogenous cell.
(Scale bars: a = 20 μm; b & c = 10 μm.)

mononematous, mainly unbranched, erect, 9–14-septate, smooth, brown to dark brown 114–273(–165.8) × 6–9(–7) µm. Conidiogenous Cells monoblastic, slightly lageniform to subulate, determinate 37–40 × 1.25–2 µm. Conidiogenous loci complanate, apical. Conidium Secession schizolytic. Conidia helicoid, 1–1½-coils, solitary, 84–150(–335) µm high, 28–39 µm wide in the coiled part, individual filaments 14–16 µm in the broadest part, 9–18 (– 34)-septate, rostrum 26–100(– 253) µm high, brown, paler towards the apex up to almost sub-hyaline, smooth, sometimes verrucose at basal part, dry. Teleomorph unknown.

Comments: The main differences between this new genus and *Circinoconis* lie in conidiophore morphology and pattern of conidiogenesis.

Circinoconis paradoxa Boedijn, the type and only species of that genus, bears conidiophores that are always trichotomously apically branched. The two lateral branches are fertile and light brown, while the central one is sterile and subhyaline. Fertile branches have integrated conidiogenous cells with percurrent proliferations (Ellis 1971). Conidia are racquet-shaped with a twisted dark brown basal part and a short to more or less rostrate paler apex. Conidiophores of *Circinoconiopsis* are, in comparison, mostly simple or rarely two-branched, but in that case the branching is neither apical nor trichotomous.

From the original description of *Circinoconis* the pattern of conidiogenesis is not clear. It was described thus: "... the development of the conidia starts with a more or less horizontally directed, nearly hyaline thread ... which forms one coil and at the point of contact the cell walls are attached to each other, giving rise to a closed ring" (Boedijn 1942). Nothing was said about the conidiogenous cells or the mode of origin of conidia (for example, whether holoblastic or enteroblastic), nor about secession. Ellis (1971) provided more information, although it is not clear whether Ellis examined the type material (the only specimen in the collection where Ellis worked, IMI 45731a, was collected on *Imperata arundinacea* from Malaysia, but information for that number, added to the IMI accessions book on 31 May 1951, does not include date of collection or any indication that it might be part of the type). He described conidiogenous cells as "... monoblastic, integrated, terminating branches, percurrent, cylindrical" Although conidiogenous cells are illustrated by Ellis as annellidic, as a result of successive conidial production, the term annellidic was not used. Conidia, according to Ellis (1971), are circinate and rostrate; his illustration differs from that of Boedijn regarding conidial morphology and shows conidia that are not ringed. In *Circinoconiopsis*, by comparison, conidiogenous cells are determinate, acutely pointed, and sub-lageniform in shape.

Conidiogenesis in this new genus follows the holoblastic pattern, and no new conidia are produced after the conidium detaches. The conidiophore branching pattern differ absolutely in the two genera: in *Circinoconis* branches are always

present in apical position and are associated with conidial production, while in *Circinoconiopsis* branches are not always present and, when they occur, are neither apical nor related to the conidiogenesis process.

Regarding conidial shape, although in both genera conidia are circinate and rostrate, in *Circinoconis* only one coil is completed and a laterally protruding cell is observed just before the beginning of the beaked part, while in *Circinoconiopsis*, conidia are one to one and half coiled and the beaked part is narrower, ending in a longer and thinner rostrum than in *Circinoconis*. The conidial coil (40–60 µm) is wider in *Circinoconis* than in *Circinoconiopsis*.

These remarkable differences between these very close genera are, in the opinion of the author, enough to justify the establishment of a new genus.

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Rediscovery of *Pseudocolus garciae* in southern Brazil

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Abstract —*Pseudocolus garciae* is described and illustrated based on fresh specimens collected in southern Brazil. This is the third known report for the species since its discovery in 1895. This clathraceous fungus is characterized by the white receptacle, which differs from the pinkish to red receptacle of *P. fusiformis*. A detailed description accompanies photographs, SEM images, and line drawings.

Key words — gasteromycetes, *Phallales*, subtropical fungi, taxonomy

Introduction

Pseudocolus Lloyd is characterized by a shortly stipitate receptacle with three to four unbranched columns bearing the slimy gleba in their internal surface, which are connected at the apex or seldom become free (Dring 1980). The genus is a poorly known due to the scarcity of collections and is regarded as one of the most difficult phalloid genera to treat satisfactorily at the species level using morphological features (Dring 1980). *Colus* Cavalier & Séchier is similar but differs in the receptacle, which is composed of columns forming an apical lattice (Dring 1980). *Pseudocolus* (*Clathraceae*, *Phallales;* Hosaka et al. 2006) currently comprises two species: *P. fusiformis* (E. Fisch.) Lloyd, with a pinkish to red receptacle widely distributed in Australasia, Europe, and the Americas (Wright 1960, Burk 1978, Dring 1980, Hemmes & Desjardin 2009, Akata & Doğan 2011), and *P. garciae*, with a white receptacle and a restricted distribution in the subtropical zone of southern Brazil (Möller 1895, Braun 1932, Rick 1961). The present paper reports the occurrence of *P. garciae*, a rare and poorly known clathraceous fungus, almost 120 years after its discovery. It follows recent observations on south Brazilian phalloid fungi (Cortez et al. 2011a, 2011b, 2011c, Trierveiler-Pereira et al. 2009).

Materials & methods

Specimens were gathered in a *Eucalyptus* plantation in Minas do Camaquã, located in the municipality of Caçapava do Sul, southeast region of the state of Rio Grande do Sul, southern Brazil (30°54′S, 53°25′W). The relict native vegetation in the area, typical of the Campos Biome of southern South America, is composed of grasses and shrubs of several families, such as *Asteraceae*, *Cyperaceae*, *Leguminosae*, and *Poaceae* (Overbeck et al. 2007), but some areas contain exotic tree species (mostly *Pinus* and *Eucalyptus*).

Fresh basidiomata were collected and analyzed macro- and microscopically in accordance to Miller & Miller (1988). Color terminology follows Kornerup & Wanscher (1978). Microscopic analysis of basidiomata comprised measurements of 20 basidiospores and hyphae (from the receptacle and rhizomorphs). For scanning electron microscopy (SEM) study, dried material was mounted directly on aluminum stubs and coated with a 5 nm thick layer of gold using a Balzers SCD 050 Sputter. Stubs were examined in a Jeol JSM-6360 LV scanning electron microscope at the Universidade Federal do Paraná (UFPR), Centro de Microscopia Eletrônica (CME). Specimens are deposited in UFRN-Fungi Herbarium (Holmgren & Holmgren 1998).

Taxonomy

Pseudocolus garciae (Möller) Lloyd, Mycol. Notes 28: 358. 1907. FIGS 1–9

≡ *Colus garciae* Möller, Brasil. Pilzb.: 146. 1895.

IMMATURE BASIDIOMATA (mycoeggs) 5–8 mm diam., white (1A1) to yellowish white (4A2); globose to subglobose, opening by splitting from the apex and forming three slightly irregular lobes. MATURE BASIDIOMATA 26–35 mm high when expanded. RECEPTACLE 9–13 × 3–4 mm, yellowish white (4A2) to orange white (5A2) throughout; with a long-cylindrical, rugose and hollow pseudostipe, splitting upwards into three tapered columns connected at their tips. COLUMNS with one large tube occupying an entire half of the inner face and a group of about three small tubes, forming the outer portion, which carry a pair of flanges running longitudinally down the arm. VOLVA membranous, 5–7 mm diam., white (7A1), with numerous white and branched (7A1) rhizomorphs projecting from the base. GLEBA olive (1F7), produced over the inner surface of the columns. ODOR fetid, resembling decaying fish. BASIDIOSPORES 3–4.5 (–5) × 1–1.5 µm, ellipsoid to bacilliform, smooth, thin-walled, hyaline in 3% KOH; surface smooth with SEM. RECEPTACLE composed of pseudoparenchymatous hyphae, 23.5–37 × 13.5–20 µm, globose to subglobose, thin-walled. CLAMP CONNECTIONS present. BASIDIA not observed. RHIZOMORPHS constituted by hyaline, thin-walled hyphae, 3–12 µm diam., smooth or encrusted by numerous angular crystals 1.5–5 µm diam., slowly dissolving in 3% KOH. Other hyphae present smooth thick walls, up to 1.5 µm diam., with brown contents, 2–3 µm diam.; terminal hyphae rounded.

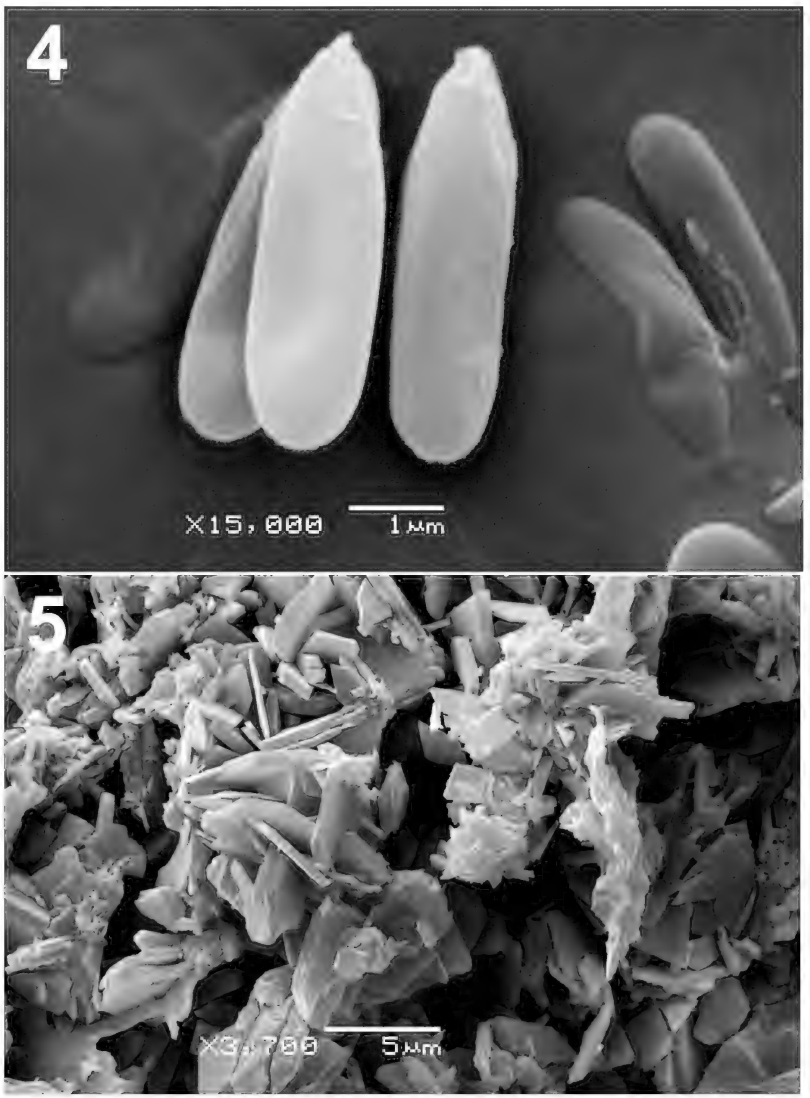
HABITAT: Saprophytic on litter and soil, in *Eucalyptus* plantation.

DISTRIBUTION: only known from southern Brazil, states of Santa Catarina (Möller 1895) and Rio Grande do Sul (Braun 1932, Rick 1961).

Figs 1–3. *Pseudocolus garciae*. 1. Fresh mature basidioma. 2. Mycoegg in cross-section. 3. Immature and mature specimens showing abundant rhizomorphs. Scale bar: 10 mm.

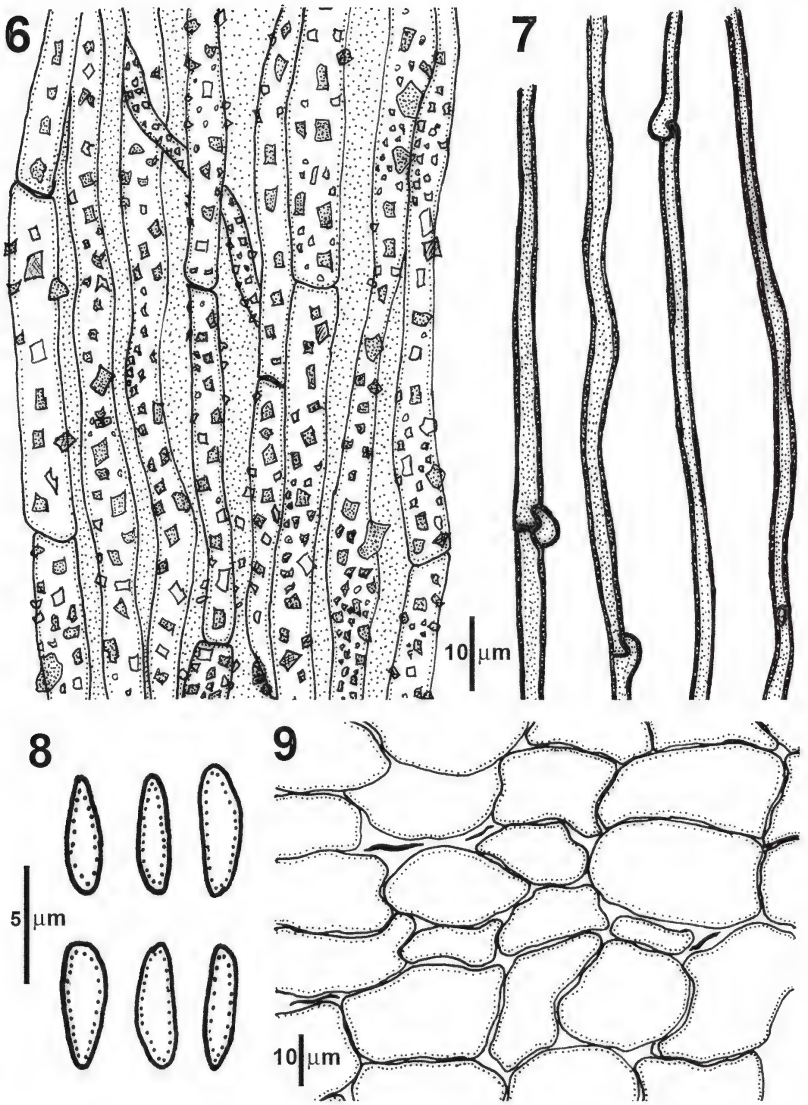
Examined specimens: **BRAZIL. Rio Grande do Sul, Caçapava do Sul,** Minas do Camaquã, 03.XII.2009, leg. M.A. Sulzbacher 209 (UFRN-Fungi 1522).

Comments: Möller (1895) originally described *P. garciae* (as a *Colus* species) from the Atlantic rainforest of Southern Brazil. The holotype was collected near



Figs 4–5. *Pseudocolus garciae* SEM photographs.
4. Basidiospores. 5. Rhizomorph surface covered by crystals.

Blumenau, state of Santa Catarina. Lloyd (1907) later transferred the species to the recently proposed genus *Pseudocolus*, but others have also considered it a *Lysurus* (Hennings 1902) or *Anthurus* (Cunningham 1931). The receptacle tubular structure of *Pseudocolus* is a good character to distinguish the genus within the anthuroid fungi (Dring 1980).



Figs 6–9. *Pseudocolus garciae*. 6. Rhizomorphs: external hyphae bearing crystals. 7. Rhizomorphs: internal hyphae. 8. Basidiospores. 9. Pseudoparenchymatic hyphae of the receptacle.

Since the original description, little information on this species has been available. In fact, the only known specimen except for the type collection was gathered in 1905 by J. Rick, reported later by Braun (1932) and Rick (1961). It is important to note that Dring (1980) translated his description from the

original protologue by Möller (1895), and both Lloyd (1907) and Braun (1932) reproduced the holotype photos. Thus, the present study reports the third collection for the species.

Our specimens fit in all aspects the descriptions provided by Möller (1895) and Braun (1932). There is no doubt the fungus is rare, since it is known only from the region in which the type collection was originally described. The new collection's habitat, however, differs from the original. Examination of new fresh material allowed observation of both immature and mature specimens (Figs 1–3) and provided new morphological data not described elsewhere: long and abundant white rhizomorphs present at the base of the mycoeggs (Fig 3), as well as the hyphal structure forming them (Figs 5–7).

SEM analysis revealed that hyphae in the rhizomorphs are covered by numerous prismatic to irregular shaped crystals (Figs 5–6) and confirmed that the basidiospore surface is, in fact, smooth (Fig 4). *Pseudocolus* comprises two species, both occurring in southern Brazil: *P. fusiformis* and *P. garciae*. In *P. fusiformis*, the receptacle is pinkish to red, the stipe is hollow and divided into 3 or 4 vertical columns tapering upwards, and the basidiospores are elliptical, 3–4 × 1–2 µm (Dring 1980). The preserved material of *P. garciae* (Brazil. Rio Grande do Sul: São Leopoldo, 1905, leg. J. Rick, PACA 12816, as *Colus garciae*) is in poor condition and therefore taxonomically inconclusive. However, judging from the descriptive notes by Braun (1932) and Rick (1961), it fits well with Möller's fungus. The present finding expands the morphological knowledge of *P. garciae* from Brazilian subtropics. Additional phylogenetic studies are needed to clarify the systematics of the Neotropical *Phallales*.

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Coccomyces pinicola sp. nov. on *Pinus armandii* from China

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Abstract —A new species of *Coccomyces*, *C. pinicola* on twigs of *Pinus armandii*, is described from Yunnan Province, China. *Coccomyces pinicola* differs from other species on twigs of *Pinus* by its much smaller and stalked ascomata. A phylogenetic analysis based on ITS rDNA sequences indicates that *C. pinicola* is more closely related to *Colpoma quercinum* (but only with weak support) than to any *Coccomyces* spp. included in the analysis.

Key words —*Ascomycota*, *Rhytismataceae*, taxonomy, phylogeny

Introduction

Coccomyces De Not. is the second largest genus in *Rhytismatales*, with over 100 species (Hawksworth 2008). Molecular analyses have shown that *Coccomyces* is polyphyletic, and that the type species, *C. tumidus* (Fr.) De Not., is not closely related to most *Coccomyces* species but to *Lophodermium eucalypti* (Rodway) P.R. Johnst. (Lantz et al. 2011). However, the current out-dated and unsatisfactory systematics of *Rhytismatales* must be used until its phylogenetics are revised.

There are seven *Coccomyces* species recorded on twigs of *Pinus* L. (Sherwood 1980, Lin et al. 1994, Hou & Piepenbring 2007). We recently collected another *Coccomyces*-like specimen on twigs of *Pinus armandii* Franch. with a unique combination of morphological characters that we propose as a new species.

Materials & methods

Morphological methods

Material with mature ascomata was selected for morphological observation. External shape, size, color, mechanism of opening of ascomata and conidiomata, as well as the characteristics of zone lines, were observed under the dissecting microscope. The detailed methods of morphological studies followed Hou et al. (2009).

Table 1. ITS rDNA sequences used in molecular studies

Species (Voucher specimen)	Sequence
Coccomyces australis	EF191240
C. dentatus	DQ491499
C. guizhouensis (HOU439A)	JX317677
C. guizhouensis (HOU439B)	JX317678
C. leptideus	GU138727
C. pinicola (HOU486A)	JX317676
Colpoma quercinum	U92306
Cudonia sichuanensis	AF433147
Dothidea sambuci	AY883094
Elytroderma deformans	AF203469
Hypoderma rubi	GU138750
Lirula macrospora	HQ902159
Lophodermium agathidis	AY100662
L. nitens	AY100640
L. piceae	AY775683
L. pinastri	AY100649
Meloderma desmazieresii	AF426056
Meria laricis	U92299
Pezicula carpinea	AF141197
Potebniamyces pyri	AY608642
Rhytisma acerinum	GQ253100
R. salicinum	AY465515
Spathularia flavida	AF433154
Soleella chinensis	GU138755
Terriera minor	AY100664
Therrya fuckelii	JF793672
T. pini	JF793676
Tryblidiopsis pinastri	JF268769
T. pinastri	JF793678

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from ascomata following the protocol of Hou et al. (2009). Three new sequences for DNA regions nuclear ribosomal DNA large subunit (nrLSU), internal transcriber spacer (ITS) and mitochondrial DNA small subunit (mtSSU) were obtained for *Coccomyces pinicola* (HOU486A) and *Coccomyces guizhouensis* Y.R. Lin & B.F. Hu (HOU439A and 439B). The LR0R/LR5 primers was used for nrLSU, and the ITS1-f/ITS4 primers for ITS (Vilgalys & Hester 1990, White et al. 1990); and the mrSSU1/mrSSU3R primers were used for mtSSU (Zoller et al. 1999). The PCR products were purified, sequenced and edited by Invitrogen Biotechnology Co. Ltd. (Beijing, China). The other ITS sequences included in this study were downloaded from GenBank (Table 1).

Phylogenetic analyses

We inserted mtSSU and nrLSU DNA sequences obtained from our samples into the matrix by Lantz et al. (2011) in order to determine the initial phylogenetic position.

The preliminary phylogeny grouped both *Coccomyces pinicola* and *C. guizhouensis* were placed in the core clade, with *C. pinicola* closely related to *Colpoma quercinum* (Pers.) Wallr. and *C. guizhouensis* close to *C. strobi* J. Reid & Cain and *Therrya* sp., although both clades had very low support (not shown). We next analyzed ITS rDNA sequences from our samples and related rhytismatalean species recorded on conifer twigs recently deposited in GenBank (Solheim et al. 2012) for additional preliminary information. We aligned the ITS rDNA dataset with Clustal X (Thompson et al. 1997) and then manually corrected by eye in Se-Al v.2.03a (Rambaut 2000). Ambiguously aligned regions were excluded from further analyses. The sequence data were first prepared and analyzed with maximum parsimony using PAUP* 4.0b10 (Swofford 1998). *Dothidea sambuci*, *Pezicula carpinea*, *Meria laricis* and *Potebniamyces pyri* were chosen as outgroup based on Lantz et al. (2011). The analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping and no MAXTREE limit. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap analysis was performed with 1000 replicates, random additions, MAXTREES set to 1000, and TBR branch swapping. For the Bayesian analysis MrModeltest 3.7 with the Akaike information criterion (AIC) was used to choose the substitution model for the separate dataset (Nylander 2004). The model GRT + I + G was chosen for the ITS sequences. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001, Ronquist & Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% of trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority consensus of the remaining trees.

Results

Molecular phylogenetics

The ITS rDNA matrix included 29 taxa. The ITS rDNA sequence alignment comprised 540 characters, with 233 phylogenetically informative positions. The maximum parsimony analysis produced one most parsimonious tree (Fig. 1) with a length (TL) = 1210 steps, consistency index (CI) = 0.4802, retention index (RI) = 0.4844, homoplasy index (HI) = 0.5198 and rescaled consistency index (RC) = 0.2326. Phylogenetic analysis supported the *Rhytismatales* (excluding *Meria laricis* and *Potebniamyces pyri*) as a clade (BP=87). This main clade was further divided into three poorly supported clades. *Coccomyces pinicola* clustered weakly with *Colpoma quercinum*, and they were sister to a moderately supported clade composed of bark-inhabiting species, *Coccomyces guizhouensis*, *Therrya fuckelii* (Rehm) Kujala, *Therrya pini* (Alb. & Schwein.) Höhn., and *Tryblidiopsis pinastri* (Pers.) P. Karst. (see Fig. 1.). The remaining clades in the phylogenetic tree were similar to a previous study (Lantz et al. 2012).

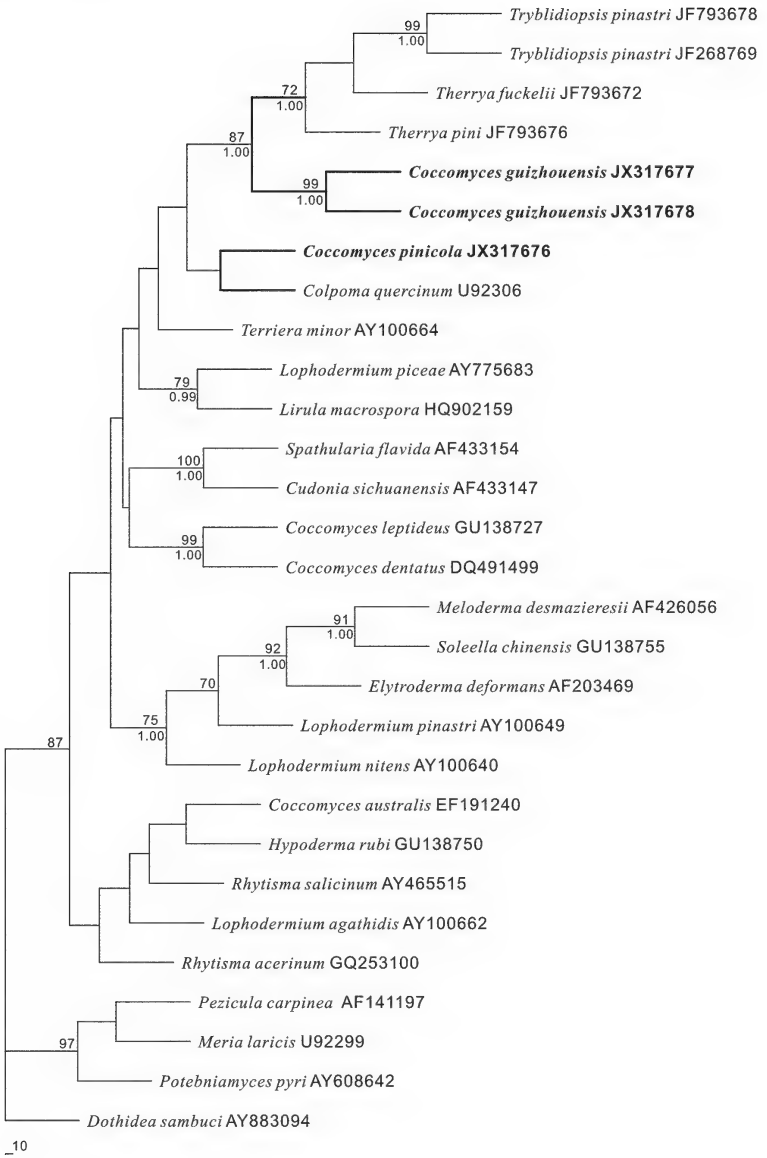
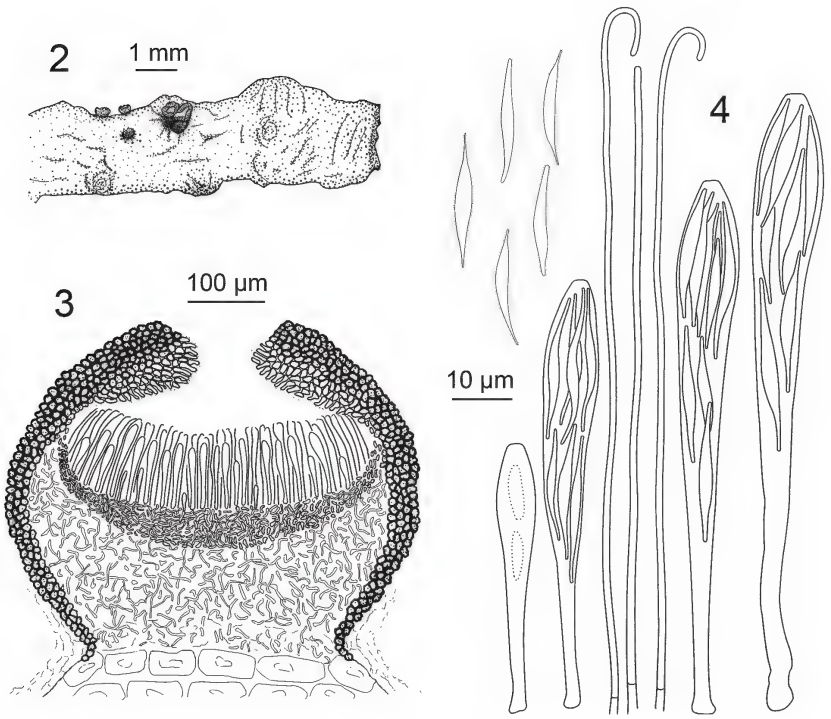


FIG. 1. Phylogenetic hypothesis derived from maximum parsimony analysis of the ITS rDNA sequences of rhytismatalean species and other related species, using *Dothidea sambuci, Meria laricis, Pezicula carpinea* and *Potebniamyces pyri* as outgroup. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches. Bayesian posterior probabilities (PP) were estimated and clades with PP>0.95 are marked under the branches.



Figs 2–4. *Coccomyces pinicola*. 2. A twig bearing ascomata. 3. Ascoma in median vertical section. 4. Paraphyses, asci with ascospores and discharged ascospores.

Coccomyces pinicola R.H. Lei & C.L. Hou, **sp. nov.** Figs 2–9

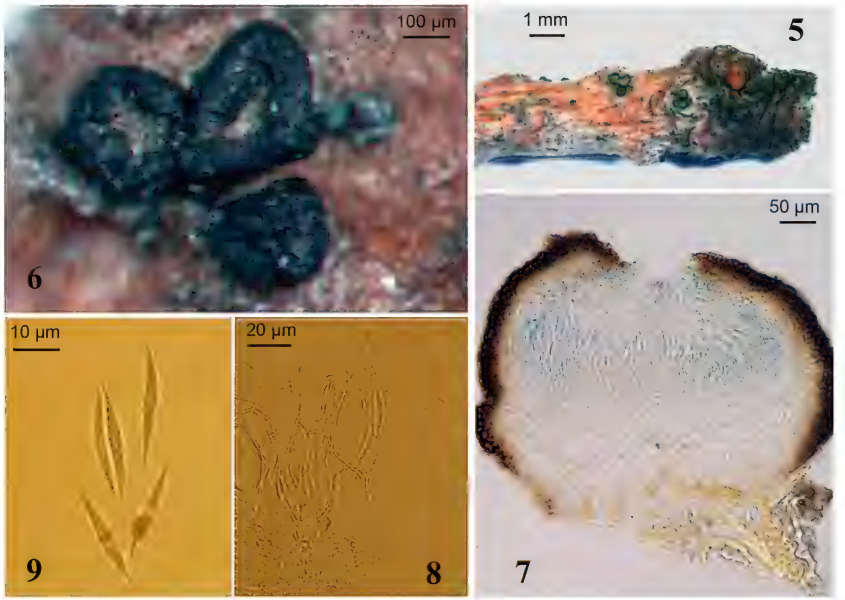
MycoBank 800963

Differs from *Coccomyces guizhouensis* by its smaller, stalked, black ascomata.

Type–China, Yunnan Province, Lijiang, Tiejiashan, alt. ca. 2000 m, on dead and dying twigs of *Pinus armandii*. 11 July 2007, C.L. Hou 486A (**Holotype,** BJTC 201202; GenBank, JX317676); C.L. Hou 486B (**Isotype,** BJTC 201212).

Etymology–*pinicola*, referring to the host genus *Pinus*.

Ascomata erumpent from the bark, stalked, circular, or slightly irregular, 200–400 µm diam., 350–500 µm high, single, occasional coalescing, with a black and crimpled surface, opening by irregular splits to expose a slightly yellow hymenium. Lips absent. In median vertical section, covering stroma 70–100 µm thick near the center of ascomata, and thinner towards the edge, consisting of an outer layer of 3 rows of dark brown, thick-walled angular cells and an inner layer of 2–4 rows of hyaline, thin-walled angular cells. Basal stroma well developed, ≤100 µm thick, consisting of short, hyaline hyphae. Subhymenium



Figs 5–9. Micrographs of *Coccomyces pinicola*. 5. A twig bearing ascomata. 6. Ascomata observed under a dissecting microscope. 7. Ascoma in median vertical section. 8. Paraphyses asci, and ascospores. 9. Discharged ascospores.

40–50 µm thick, consisting of small, hyaline cells. Paraphyses 80–130 × 1 µm filiform, not branched, often circinate at the apex. Asci ripening sequentially, thin-walled, cylindrical-clavate, 60–110 × 7–11 µm, with a 30–40 µm long stalk, truncate at the apex, without circumapical thickening, J–, 8-spored. Ascospores 25–30 × 2–3 µm, long-fusiform, acute at both ends, hyaline, aseptate, with a thin or indistinguishable gelatinous sheath.

Conidiomata and zone lines not observed.

Distribution. *Coccomyces pinicola* is known only from the type locality, Yunnan, China.

Discussion

There are seven *Coccomyces* species recorded on twigs of *Pinus*, namely *C. cembrae* Rehm, *C. guizhouensis*, *C. irretitus* Sherwood, *C. lijiangensis* C.L. Hou & M. Piepenbr., *C. papillatus* Sherwood, *C. parvulus* Sherwood, and *C. strobi*. *Coccomyces pinicola* is distinguished from these other species on *Pinus* twigs by its much smaller ascomata with a conspicuous stalk. The asci and ascospores of *C. pinicola* are similar to those of *C. guizhouensis*, a species that also occurs on *Pinus armandii*, but *C. guizhouensis* has much larger, discoid ascomata ≤1.5 mm diam (Lin et al. 1994). Furthermore, host tissue tightly covers the *C. guizhouensis* ascomatal surface, resulting in a grey to dark grey ascomatal

surface in contrast to *C. pinicola* where the host tissue on the covering layer is detached and the black ascoma is exposed. The other *Coccomyces* species on *Pinus* are easily distinguished by their more or less filiform ascospores. Asci and ascospores of *C. pinicola* also resemble *Therrya abieticola* C. L. Hou & M. Piepenbr. on *Abies*. However, the *T. abieticola* ascomata are inconspicuously discoid in surface view and the paraphyses are typical of *Therrya*, i.e. filiform paraphyses with apical knobs.

The combined nrLSU and mtSSU DNA sequence analyses by Lantz et al. (2011) indicated that a *Therrya* sp. and *Coccomyces strobi*, both occurring on *Pinus* twigs, formed a weak clade, which weakly groups with an adjacent clade containing *C. crystalligerus* Sherwood and *Colpoma quercinum. Tryblidiopsis pinastri* on *Picea* twigs weakly groups with *Cudoniaceae* species (Lantz et al. 2011). In the present ITS rDNA sequence analysis, *Tryblidiopsis pinastri* is not closely related to the monoclade of *Cudonia sichuanensis* and *Spathularia flavida*, but to a strongly supported clade composed of most bark-inhabiting species on conifers, *Coccomyces guizhouensis*, *Therrya fuckelii*, and *Therrya pini.* Interestingly, the new species *Coccomyces pinicola* is distally related to *C. guizhouensis* but weakly sister to *Colpoma quercinum,* which occurs on the bark of *Quercus* and has larger elliptic ascomata opening by a longitudinal split and filiform ascospores. The results of the phylogenetic analysis was inconsistent with the morphological characters and it cannot be explained clearly now.

Our molecular analysis suggests that bark-inhabiting species on conifers (except *C. pinicola*) are genetically highly related, although they differ in many aspects, such as ascoma shape and opening patterns and ascospore characters. Lantz et al. (2011) considered that ascospore characters and ascoma shapes are limited for generic delimitation and suggested that *Therrya, Hypohelion*, *Coccomyces* (excluding the type species *C. tumidus*), *Colpoma*, and *Duplicariella* be recombined in the future. Our current analysis indicates that *Tryblidiopsis* might also belong in this group.

Acknowledgments

We are grateful to Prof Ying-Ren Lin and Prof. Ming Ye for serving as pre-submission reviewers, to Joanne E. Taylor for her assistance with English language, and to Dr. P.R. Johnston for giving valuable suggestions for the phylogenetic position of the new species. The study was supported by the National Natural Science Foundation of China (No. 30870014, 31170019 and 31270065) and PHR (KZ201110028036).

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Sporormiella octomegaspora, a new hairy species with eight-celled ascospores from Spain

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Abstract — An ascolocular ascomycete with semi-immersed, hairy and pyriform pseudothecia, abundant pseudoparaphyses, fissitunicate 8-spored asci, and dark, very large 8-celled ascospores has been isolated from deer dung in Andalusia (Spain). Based on morphological features, a new species is erected and accommodated in *Sporormiella*, which the authors regard as a genus independent of *Preussia*. The new species is discussed and placed in a key, and a previous worldwide key to *Sporormiella* species with 8-celled spores is updated.

Key words — coprophily, phylogeny, *Pleosporales*, relationships, *Sporormiaceae*

Introduction

Sporormiella Ellis & Everh. (*Sporormiaceae* Munk, *Pleosporales*) is characterised by ascoloculate, aperiphysate, ostiolate pseudothecia, fissitunicate, elongated, 8-spored asci with a scarcely developed apical apparatus, dark colored, transversely septate, 4- to poly-celled ascospores with germ-slits and usually with a gelatinous envelopment, and preferable growth on dung (Ellis & Everhart 1892, Ahmed & Cain 1972, Barr 2000).

Preussia Fuckel, in the same family, has morphological features so similar to *Sporormiella* that its independence has been questioned. We refer to previous works on this subject (Doveri 2004, 2005, 2007, 2011; Doveri & Coué 2008) to explain why the senior author regards *Sporormiella* as distinct from *Preussia* (Cain 1961; Ahmed & Cain 1972; Barrasa & Checa 1991; Lumbsch & Huhndorf 2007, 2010; Kirk et al. 2008). That view has been strengthened by an extensive phylogenetic study on *Sporormiaceae* (Kruys & Wedin 2009), which does not definitely resolve the question of synonymy.

Another view (von Arx 1973, Guarro et al. 1997a, Chang & Wang 2009) that regards *Sporormiella* as a later synonym of *Preussia* has led to the recombination of several *Sporormiella* species in *Preussia* (von Arx 1973; Valldosera & Guarro

1990; Guarro et al. 1997a,b; Abdullah et al. 1999; Arenal et al. 2004, 2005, 2007; Chang & Wang 2009, Kruys & Wedin 2009) or even the description of new *Sporormiella* species in *Preussia* sensu lato (Arenal et al. 2005, 2007; Chang & Wang 2009, Asgari & Zare 2010).

Sporormiella encompasses, at the present, more than 80 species, mostly with glabrous pseudothecia and 4-celled ascospores, a minority (12) with hairs, and/or more than 4-celled ascospores, very few with ascospores consisting of a variable number of cells (2–10).

A recent collection from Andalusia (Spain) of a hairy species with consistently 8-celled ascospores has been submitted to our observation. Its morphological features are unique, thus allowing us to recognise it as a new *Sporormiella* species.

The aim of our study is to define the new species and establish its relationships with other hairy *Sporormiella* spp. and a group of *Sporormiella* with 8-celled ascospores.

Materials & methods

Dried and fresh specimens of *Sporormiella octomegaspora* were sent us from Spain, the fresh material still on small pieces of red-deer (*Cervus elaphus*) dung. Fresh material (five specimens) was utilised for macro- and microscopic studies and for attempting, unsuccessfully, isolation in axenic culture. The specimens were picked from the dung with a sterile needle, washed in distilled water, and placed in a drop of water on a microscope slide. Microscopic examinations were carried out on specimens mounted in water, Melzer's reagent, and methyl blue. Spore size was measured in water and calculated on 50 ascospores discharged from mature asci in 4 ascomata. The collection has been preserved as dried material and slides (MCVE). Herbarium abbreviation follows Holmgren & Holmgren (2012).

Sporormiella spp. that have not been recombined in that genus, are indicated by presenting the current binomials with the genus enclosed in quotation marks (e.g., "*Sporormia*" *carpinea*, "*Preussia*" *variispora*).

Taxonomy

Sporormiella octomegaspora Doveri & Sarrocco, **sp. nov.** Pl. 1–3

MycoBank 803276

Differs from all other *Sporormiella* species by its larger 8-celled ascospores.

Type: Spain, Andalusia, Huelva, Doñana National Park (approx. 37°N 6.5°W), on red deer dung, 28.10.2008, leg. A. Suárez (**Holotype**, MCVE 27409)

Etymology: From the Greek *octo* = eight, *megas* = large, *spora* = spore, referring to the large, 8-celled ascospores

Macrocharacters — pseudothecia 720–900 × 400–500 µm, ostiolate, pyriform, semi-membranous, dark brown to blackish, rough, tomentose owing to a dense net of hyphoid hairs spread all over the ascoma. Neck differentiated, 200–250 × 180–200 µm, cylindrical, somewhat darker, subcoriaceous, with the

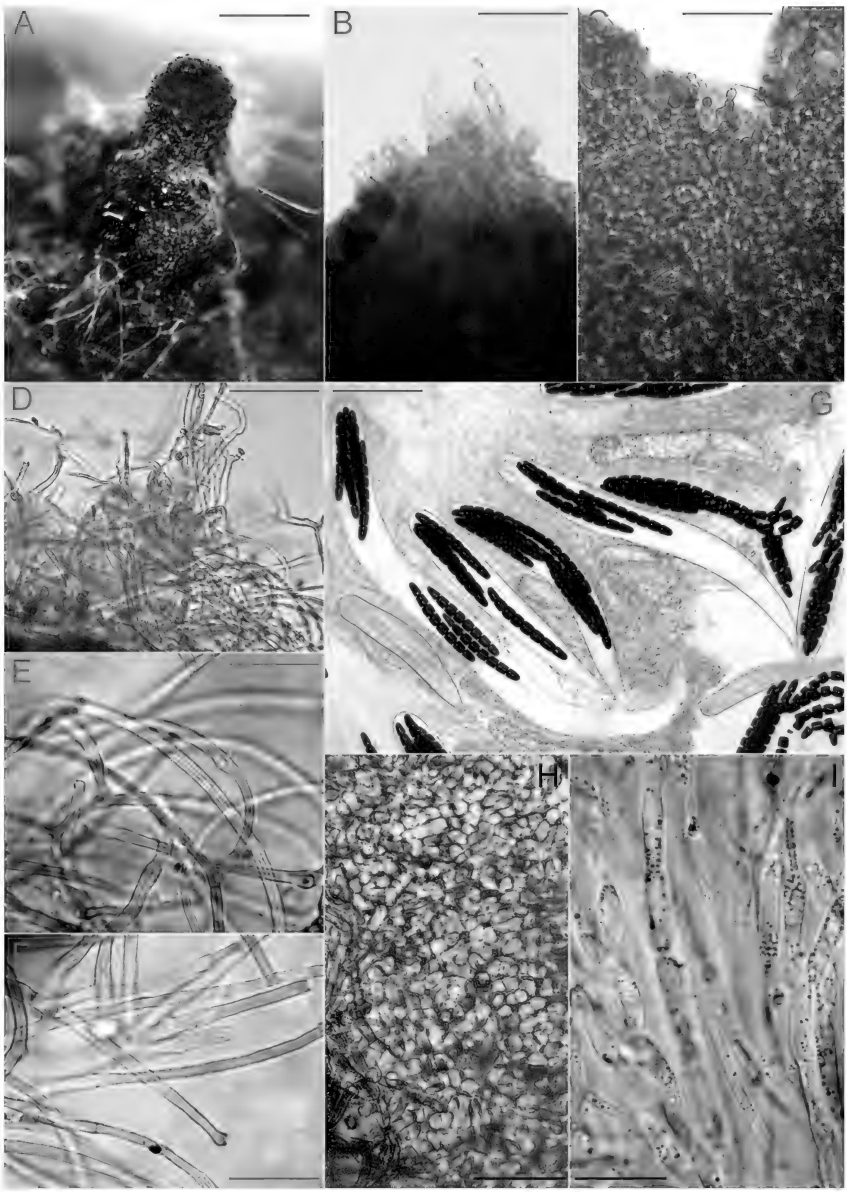

Plate 1. *Sporormiella octomegaspora* (holotype): A = ascoma on dung; B = hairs at the neck apex; C = detail of exoperidium at the neck; D–F = peridial hairs; G = centrum; H = detail of exoperidium; I = Pseudoparaphyses. Scale bars: A = 300 µm; B, E–F = 20 µm; C = 80 µm; D, H = 40 µm; G = 150 µm; H = 25 µm.

same net of hairs at its base and shorter, more isolated hairs at the apex, hardly observable at low magnification (10 ×).

MICROCHARACTERS — PERIDIUM pseudoparenchymatous, two-layered: endostratum of pale brown to almost hyaline, thin-walled, polygonal cells, 13–18 × 12–15 µm, exostratum a textura angularis of dark brown, thick-walled, polygonal cells, 6–10 × 5–8 µm, with intervals of cylindrical cells in rows (textura prismatica), a textura globulosa-angularis of blunt angular to roundish and somewhat darker cells at the neck, 5–9 µm diam., papillate around the ostiole. Some carbonaceous deposits sprinkled all over, but more abundant on the neck; PERIDIAL HAIRS hyphoid, very long, wavy, strongly intertwined, fairly thick-walled, quite dark brown, septate, often branched, sometimes anastomosed and somewhat encrusted, blunt at the tips, 2–3.5 µm diam.; NECK HAIRS hyphoid at the base, similar in size and shape to peridial hairs, polymorphous, paler, stiffer and shorter at the apex, 15–30 µm long, usually with a rounded, sometimes clavate tip; PSEUDOPARAPHYSES mixed with the asci and exceeding them, cylindric-filiform, sometimes submoniliform, 3–7 µm diam., often branched, septate, usually slightly tapering upwards, somewhat constricted at the septa, containing abundant hyaline vacuoles; ASCI 325–450 × 47–72 µm, fissitunicate, inamyloid, 8-spored, cylindric-clavate to clavate, lacking an apical apparatus, roundish or dome-shaped or even slightly flattened at the apex, usually quite abruptly narrowing below in a short stalk, 12–30 µm long; ASCOSPORES (148–)152–175(–180) × 16–18(–20) µm, usually three to five bundled in the upper portion of the ascus, the others placed at various levels below, and one usually the lowest, hyaline in the early stages, pale brown later, finally dark brown, cylindric-subfusiform, individually surrounded by a broad gelatinous envelope, smooth, thick-walled, constantly 8-celled, deeply and transversely septate. Cells easily separable at any level, each with a sigmoidal, parallel germ slit. End cells conical, with blunt tips, narrower and usually longer than the middle ones, but often not longer than the second and the seventh, third to sixth cells from the upper end sub-cubical, almost as long as wide, and the third usually the broadest, sometimes slightly wider than long, the second and seventh cell cylindrical, usually longer than wide, all cells with fairly acute angles.

ECOLOGY & DISTRIBUTION—About fifteen scattered specimens on red-deer (*Cervus elaphus*) dung in the field in October. To date only known from the type locality.

Discussion

Sporormiella octomegaspora is characterised by large and densely hairy ascomata, with hairs widespread both on the pseudothecial venter and neck, large and short-stalked asci, and very large, constantly 8-celled, subfusiform ascospores with sigmoidal germ slits and a particular arrangement inside

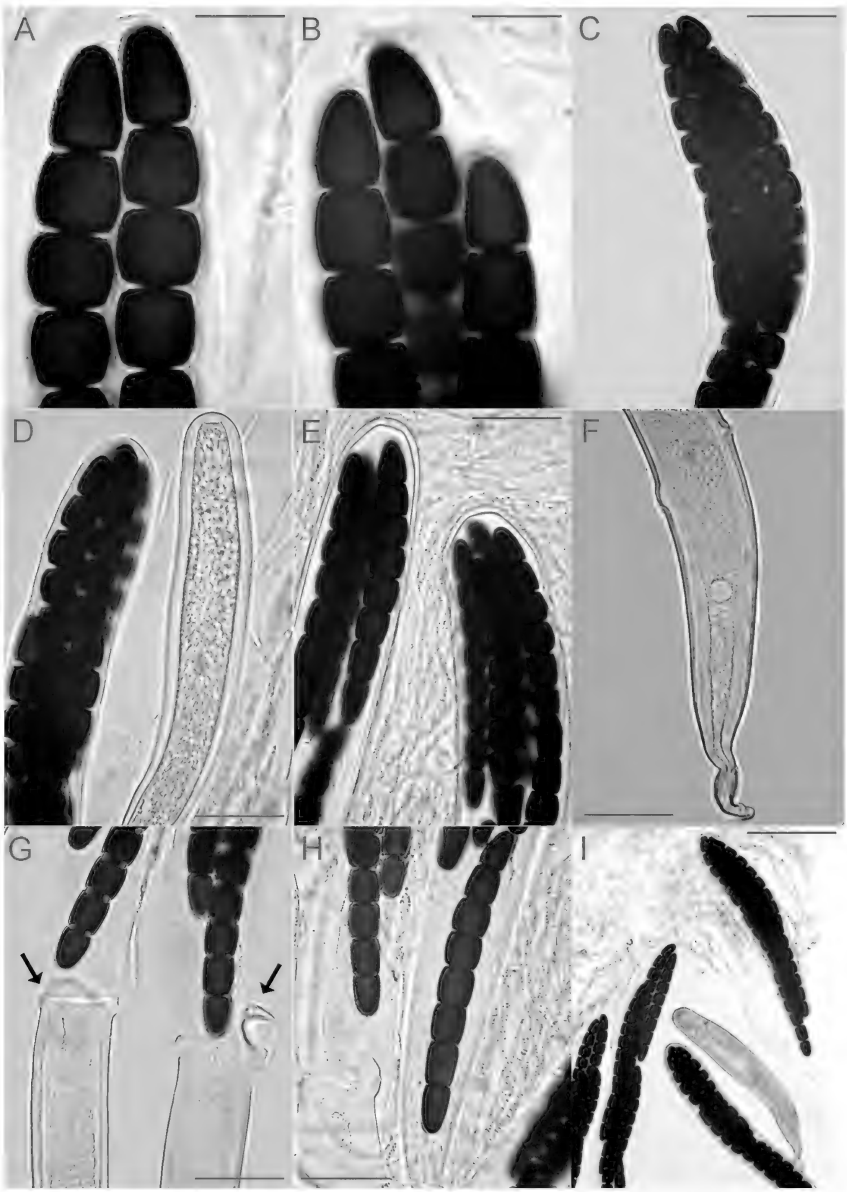

Plate 2. *Sporormiella octomegaspora* (holotype): A–B = upper cells of ascospores at the ascus apex; C-E = upper and middle parts of asci with ascospores; F = ascus base; G = detail of fissitunicate (arrows) asci; H = ascus bases with ascospores. Scale bars: A–B = 20 µm; C–H = 50 µm; I = 150 µm.

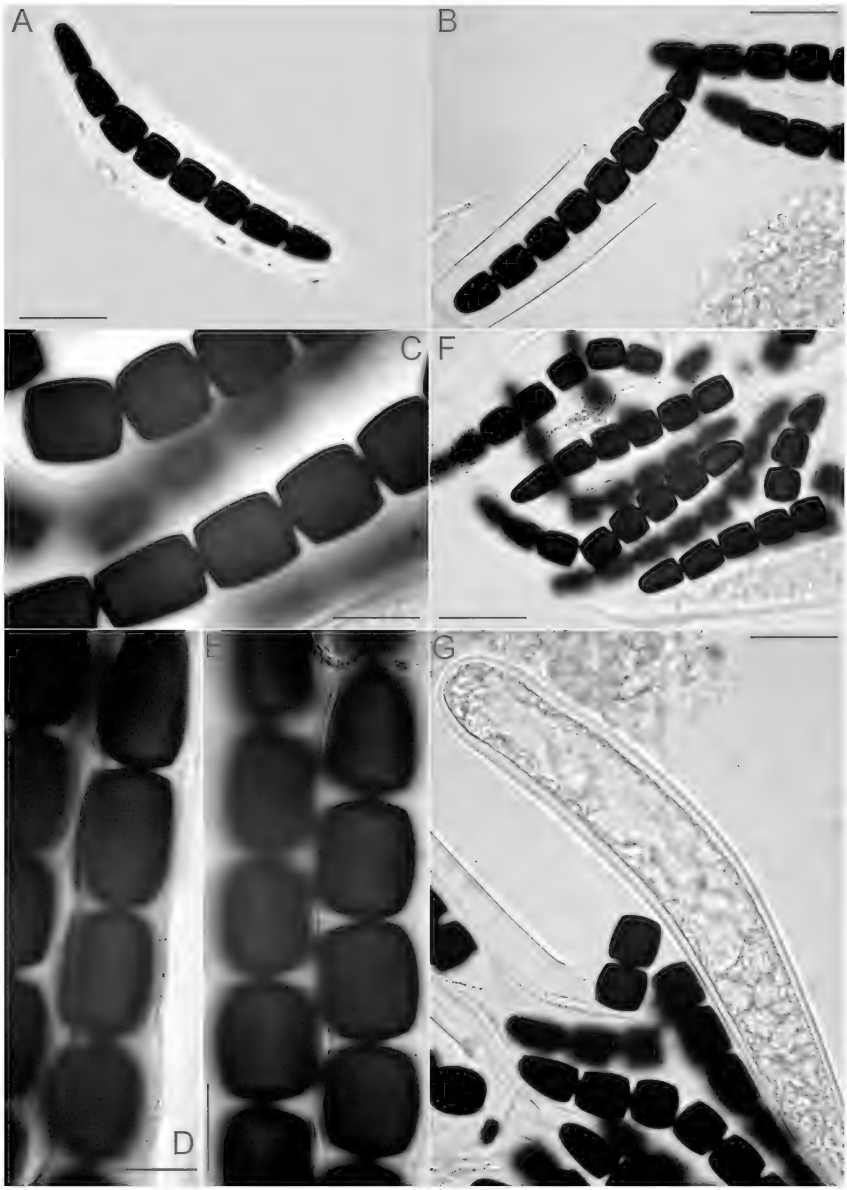

Plate 3. *Sporormiella octomegaspora* (holotype): A–B = ascospores; C–E = detail of ascospores; F–G = spore cells. Scale bars: A–B, F = 35 μm; C = 18 μm; D–E = 15 μm; G = 30 μm.

the asci. The whole of these features allows us to separate *S. octomegaspora* from species with hairy pseudothecia and/or 8-celled ascospores and easily distinguish it from all other *Sporormiella* spp.

In their basic monograph on *Sporormiella*, Ahmed & Cain (1972) reported 3 hairy species with 4-celled ascospores and 18 species with constantly 8-celled ascospores, including the coprophilous "*Sporormia*" *octoloculata* Fabre and "*Sporormia*" *pulchra* E.C. Hansen (both cited as doubtful or not examined by them) and the non-coprophilous, non-examined "*Sporormia*" *carpinea* Fautrey, "*Sporormia*" *gigaspora* Fuckel, *S. subticinensis* (Mouton) Dugan & R.G. Roberts, and *S. ticinensis* (Pirotta) Doveri (Doveri 2004). They also reported 2 species with inconsistently 8-celled ascospores, *S. commutata* (Niessl) S.I. Ahmed & Cain (7–9-celled), and "*Sporormia*" *variabilis* G. Winter (5–8-celled). All *Sporormiella* species described by Ahmed & Cain (1972) — with the exception of *S. insignis* (Niessl) S.I. Ahmed & Cain and *S. splendens* (Cain) S.I. Ahmed & Cain — whether with constantly or inconstantly 8-celled ascospores, differ from *S. octomegaspora* due to ascospores that are much shorter than 100 µm.

Sporormiella splendens has, like *S. octomegaspora*, short-stalked asci, but can be easily distinguished by its glabrous ascomata, smaller pseudothecia and asci, and somewhat shorter but notably narrower cylindrical ascospores (9–13 µm diam., Cain 1934, Ahmed & Cain 1972, Furuya & Udagawa 1972, Leenurm 1998, Treigienė 2004), with middle cells longer than broad and an oblique to diagonal germ slit.

Although it has short-stalked asci, and ascospores with cubical middle cells and an arrangement inside the asci similar to *S. octomegaspora*, *S. insignis* differs in having glabrous and much smaller pseudothecia, smaller asci (Winter 1887, Bayer 1924, Prokhorov & Armenskaya 2003), and somewhat narrower and notably shorter (100–122 × 14–15 µm; Niessl 1878, Ahmed & Cain 1972) ascospores, with middle cells equal in size to each other (so the spore shape is cylindrical rather than cylindric-fusiform) and diagonal germ slits.

Bell (2005), who collected the species in Australia on Eastern Grey kangaroo dung and which she described as *S. insignis*, straddles *S. insignis* and *S. splendens*, "having the ascospore width of the former and the ascospore length of the latter," but in our opinion drawings and measurements reported in her work match those of *S. octomegaspora*. Although the Australian material was unfortunately inadequate for a herbarium sample (Bell, pers. comm.), our examination of a slide from Australia strengthens our original opinion, as Bell's material has only slightly wider spores on average.

Doveri (2004) mentioned and briefly described or discussed (sometimes as *Preussia*) all new *Sporormiella* spp. published after Ahmed & Cain's (1972) monograph, some of which are characterised, like *S. octomegaspora*, by hairy pseudothecia and/or 8-celled ascospores. Among them, the hairy species,

S. lasiocarpa Lorenzo and "*Preussia*" *aquilirostrata* Guarro et al. (Guarro et al. 1997b), have 4-celled and much smaller ascospores [respectively 62–76 × 10–12 µm (Lorenzo 1994), 34–44 × 8.8–11.5 µm (Guarro et al. 1997b)] than *S. octomegaspora;* on the contrary, the species with 8-celled ascospores — *S. oblongiclavata* M.E. Barr & Malloch, *S. tomilinii* O.V. Korol., and "*Preussia*" *variispora* Abdullah et al. — do not have hairy pseudothecia, and "*Preussia*" *variispora* has both 7- and 8-celled ascospores, while all have much smaller ascospores (Abdullah et al. 1999, Barr 2000, Korolyova 2000) than *S. octomegaspora.*

Besides establishing new species of *Sporormiella*, Doveri (2005, 2007), Doveri & Coué (2008), and Chang & Wang (2009) updated the list of new *Sporormiella* spp. (sometimes as *Preussia*) published since 2004. None of them has consistently 8-celled ascospores.

The Welt & Heine (2007) worldwide key to *Sporormiella* spp. with 8-celled ascospores is here updated with the addition of *S. octomegaspora* and other species not previously included:

Worldwide key to *Sporormiella* species with 8-celled ascospores

[N.B.: "***Sporormia octoloculata***" and "***Sporormia***" ***carpinea*** have been omitted from the key, as the original descriptions are meagre]

1a. Ascospores 5–10-celled (never consistently 8-celled) **2**

1b. Ascospores consistently 8-celled **7**

2a. Ascospores 5–8-celled, 62–75 × 14–19 µm (Winter 1874), cylindrical, with middle cells usually broader than long ..... "***Sporormia*" *variabilis***

2b. Ascospores 7–9-celled, different in shape **3**

3a. Ascospores (7–)8–9(–10)-celled, 45–53 × 8–10 µm, fusiform with middle cells wider than long, and the third cell usually the broadest ..... ***Sporormiella* sp.** (described by Richardson 2004b)

3b. Ascospores longer, cylindric-clavate **4**

4a. Ascospores 50–60 × 8–10.5 µm (Ahmed & Cain 1972), usually 9-celled, sometimes 7–8-celled, with the middle cells broader than long, and the third cell from the upper end the broadest ..... ***S. commutata***

4b. 9-celled ascospores rare or absent **5**

5a. Ascospores 65–75 × 7–12 µm, 7–8-celled, with middle cells as long as broad, and the third cell from the upper end the largest ..... "***Preussia*" *variispora***

5b. Ascospores mostly 8-celled, sometimes 7–9-celled **6**

6a. Ascospores 70–90 × 11–13 µm, with the third or fourth cell from the upper end the largest ..... ***Sporormiella* sp.1** (described by Richardson 2004a)

6b. Ascospores 58–72 × 8–9.5 µm, cylindrical, with isodiametric cells, to cylindric-clavate, with the upper cells shorter than wide ..... ***Sporormiella* sp. 2** (described by Richardson 2004a)

7a (1b). Ascospores >100 µm long **8**
7b. Ascospores <100 µm long **10**

8a. Ascomata hairy, 720–900 × 400–500 µm. Ascospores 152–175 × 16–18 µm, cylindric-subfusiform with third to sixth cells from the upper end almost as long as wide, and the third usually the broadest. Germ slits sigmoid and parallel ..... ***S. octomegaspora***
8b. Ascomata glabrous, smaller. Ascospores narrower, cylindrical. Germ slits oblique to diagonal **9**

9a. Ascomata 550–600 × 350–400 µm. Ascospores 135–165 × 9.5–12.5 µm (Cain 1934), with middle cells longer than broad, cylindrical ..... ***S. splendens***
9b. Ascomata subglobose, 250–330 µm diam. Ascospores 100–122 × 14–15 µm (Ahmed & Cain 1972), with middle cells almost equal in size, cubical ..... ***S. insignis***

10a. (7b) Ascospores variable in shape, cylindrical to cylindric-fusoid or slightly cylindric-clavate, 50–58 × 6–8 µm, with middle cells equal in width, or the fourth and/or the fifth, or even some other cell, the broadest ... ***Sporormiella* sp.** (described by Cribb 1994)
10b. Ascospores quite constant in shape **11**

11a. Ascospores cylindrical, with middle cells almost equal in width **12**
11b. Ascospores (sub)clavate, with one broader cell **16**

12a. Ascospores more than 60 µm long **13**
12b. Ascospores less than 60 µm long **14**

13a. Ascospores 79–95 × 14–16 µm (Ahmed & Cain 1972). Middle cells broader than long and with an obliquely transverse germ slit ..... ***S. platymera***
13b. Ascospores 72 × 9 µm (Fuckel 1871), with middle cells as long as wide ..... ***"Sporormia" gigaspora***
(***S. subticinensis*** with ascospores 62–75 × 9–12 µm (von Arx & Storm 1967), is hardly separable from ***"Sporormia" gigaspora***, although Mouton (1897) noted that it differs in having clavate rather than oblong asci and much smaller ascomata)

14a (12b). Ascospores 47–57 × 12–14 µm, with middle cells usually broader than long ..... ***"Sporormia" pulchra***
14b. Ascospores narrower, with middle cells almost as long as wide **15**

15a. Ascospores 48–58 × 6–7 µm with diagonal germ slits ..... ***S. bipartis***
15b. Ascospores 40–44 × 8 µm (Pirotta 1878). Germ slits not described .. ***S. ticinensis***

16a (11b). Ascospores 30–48 × 4–7 µm, with the second cell from the upper end the broadest, and with almost parallel germ slits. Non-coprophilous ..... ***S. oblongiclavata***
16b. Third or fourth cell the broadest. Germ slits oblique to diagonal (not described in ***S. tomilinii***). Coprophilous species **17**

17a. Third cell the broadest. Cells easily separable to non-separable **18**
17b. Fourth cell the broadest. Cells hardly separable **23**

18a. Ascospores 38–43.5 × 8–9 µm (Doveri 2008) (40 × 5–6 µm, Auerswald 1868; 40–48 × 7–8 µm, Ahmed & Cain 1972). Cells easily separable from each other, and the end ones conic-ovoid. Asci gently narrowing below in a long stalk ..... ***S. octomera***

18b. Ascospores larger **19**

19a. Asci abruptly contracted in a very short stipe **20**

19b. Asci gently narrowed in a stipe. Ascospores with (sub)conical end cells **21**

20a. Ascospores 48.5–63 × 12.5–14.5 µm (Doveri 2008) (48–58 × 12–14 µm, Ahmed & Cain 1972). Spore cells hardly separable, end cells hemispheric ..... ***S. octonalis***

20b. Ascospores 52–57 × 8–9 µm (Ahmed & Cain 1972), with cells easily separable, end cells subconical ..... ***S. schadospora***

21a (19b). Ascospores 71–90 × 12–16 µm (Bommer & Rousseau 1886) (65–80 × 12–15 µm, Ahmed & Cain 1972). Asci short-stipitate ..... ***S. affinis***

21b. Ascospores shorter **22**

22a. Ascospores 52.5–60 × 9.5–10.5 µm (Doveri 2008) (50–59 × 10–11.5 µm, Ahmed & Cain 1972). Cells not separable. Asci long-stipitate .... ***S. corynespora***

22b. Ascospores 55–70 × 13–15 µm. Cells easily separable. Asci short-stipitate ..... ***S. tomilinii***

23a (17b). Ascospores 49–58 × 9–10 µm (Ahmed & Cain 1972). Asci abruptly short-stipitate ..... ***S. ontariensis***

23b. Ascospores smaller **24**

24a. Ascospores 40–51.5 × 7.5–8.5 µm (Doveri 2008) (40–49 × 8–9 µm, Ahmed & Cain 1972). Asci abruptly short-stipitate ..... ***S. pascua***

24b. Ascospores 34–42 × 7 µm (Doveri 2008) (32–36 × 5.5–6.5 µm, Ahmed & Cain 1972). Asci gently short-stipitate ..... ***S. minipascua***

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Neolinocarpon attaleae sp. nov. on *Attalea funifera* (*Arecaceae*) from Brazil

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Abstract — *Neolinocarpon attaleae* is described from the palm *Attalea funifera* collected in Brazil. This species is illustrated and compared with similar species. A key to all species of *Neolinocarpon* is provided.

Key words — *Ascomycota*, biodiversity, palm fungi, taxonomy, Brazilian mycota

Introduction

Hyde (1992) introduced the genus *Neolinocarpon* K.D. Hyde to accommodate *N. globosicarpum* K.D. Hyde, a *Linocarpon*-like specimen, with deeply immersed ascomata formed beneath a slightly raised or flattened clypeus. Since then, seven new species and a new combination have been added to *Neolinocarpon*: *N. australiense* K.D. Hyde et al., *N. calami* K.D. Hyde et al., *N. enshiense* K.D. Hyde et al., *N. eutypoides* (Penz. & Sacc.) K.D. Hyde et al., *N. inconspicuum* K.D. Hyde et al., *N. nonappendiculatum* K.D. Hyde et al., *N. nypicola* K.D. Hyde & Alias, and *N. penniseti* Bhilabutra & K.D. Hyde (Hyde et al. 1998, Hyde & Alias 1999, Bhilabutra et al. 2006). All species of *Neolinocarpon* have so far been collected on palms, except *N. penniseti*, a species from grass.

During palm microfungi surveys of Pernambuco and Bahia States along the Brazilian Northeastern coast 81 ascomycetes were identified

(Souza et al. 2008, Vitoria et al. 2008, 2010, 2011a,b, 2012). In this paper a new species, *Neolinocarpon attaleae* from *Attalea funifera* Mart. ex Spreng. (*Arecaceae*), is illustrated, described, and compared with similar species within *Neolinocarpon*.

Materials & methods

Dead leaves of palms were collected in the municipality of Una, Bahia state. The specimens collected were incorporated into CEPEC Herbarium (Mycological Collection) in Itabuna, Bahia. Observations under the stereomicroscope were followed by the study of squash preparations and vertical free hand sections of the ascomata. Morphological features were described and measured using a Zeiss-Axiophot E microscope and photographed were taken using a digital camera Cyber-shot 4.1 Mega Pixels DSC-P73x, adjusted to the eyepieces of the microscope and the stereomicroscope. All measurements were made in water preparations. Samples stained with lacto-glycerol cotton blue, Melzer's reagent and acid lactofuchsin were also prepared.

Neolinocarpon attaleae Vitória & J.L. Bezerra, **sp. nov.** PLATES 1–2

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Differs from *Neolinocarpon nonappendiculatum* by its shorter, wider, slightly curved to straight, clavate ascospores.

TYPE: Brazil. Bahia, Una, Estação Experimental Lemos Maia-CEPLAC, 15°16′207″S 39°05′532″W, on dead leaf (rachis) of *Attalea funifera*, 21.VII.2009, Nadja Vitória, 156 (**Holotype**, CEPEC 2320).

ETYMOLOGY: *attaleae,* in reference to the host genus, *Attalea.*

ASCOMATA deeply immersed in host tissue, externally visible as black, shiny, raised and spherical bodies, due its conspicuous clypeate ostioles; in vertical section 350–880 × 220–650 µm (including ostiole), subglobose with a blackened clypeus surrounding the periphysate ostiole. OSTIOLE ca 117.5–250 µm diam, 170–250 µm high, central. PERIDIUM up to 55 µm wide, composed of several layers of brown, elongated, flattened cells, sometimes comprising two darker outer strata and one lighter inner stratum. PARAPHYSES up to 5 µm wide, numerous, hypha-like, septate, unbranched. ASCI 137.5–227.5 × 7.5–14(–15) µm, 8-spored, cylindric-clavate, usually curved, sometimes straight, long pedicellate, unitunicate, apically rounded, with a refractive, discoid, non-amyloid subapical ring ca 1.5–2 × 1–2 µm. ASCOSPORES (52.5–)57.5–93(–105) × 3–4(–5) µm, fasciculate, hyaline, guttulate, unicellular, smooth, slightly curved to straight, filiform-cylindrical, filiform-fusoid to ± clavate, apex rounded, attenuated towards rounded base, with refractive septum-like bands, lacking appendages.

ADDITIONAL SPECIMENS EXAMINED — **BRAZIL. BAHIA**: Una, Estação Experimental Lemos Maia-CEPLAC, 18.XII.2008, Nadja Vitória, 154,155 (CEPEC 2318, 2319); 15.III.2010, Nadja Vitória, 157, 158 (CEPEC 2321, 2322); 06.VII.2010, Nadja Vitória, 159 (CEPEC 2323).

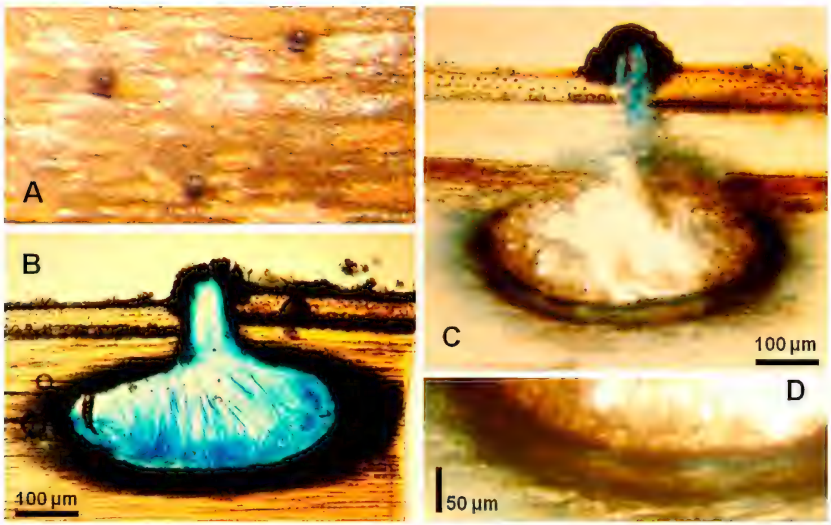

Plate 1. *Neolinocarpon attaleae* (from holotype): A. Appearance of the ascomata on the host surface. B–C. Vertical section through an ascoma. D. Section through peridium.

Comments — The collections from *Attalea funifera* were identified as *Neolinocarpon* according to Hyde (1992), but differ from other species of the genus mainly by ascospore morphology. As in *N. penniseti*, *N. inconspicuum*, and *N. nonappendiculatum*, *N. attaleae* lacks appendages at the apices of the ascospores (Bhilabutra et al. 2006). Ascospores of *N. attaleae* have similar dimensions to *N. penniseti* ((52.5–)57–64(–84) × 2.5–3 µm; Bhilabutra et al. 2006) and *N. inconspicuum* (76–98 × 2–3 µm; Hyde et al. 1998), but the new species differs in ascoma size and shape, asci and subapical ring, and ascospore morphology.

Key to species of *Neolinocarpon*

1a. Ascospores with appendage 2
1b. Ascospores without appendage 7

2a. Ascospores longer than 65 µm 3
2b. Ascospores 42–64 × 2–3.5 µm; mucilaginous pad at narrow truncated base ..... *N. enshiense*

3a. Intertidal 4
3b. Terrestrial 5

4a. Ascospores 70–119.5 × 2–3 µm; ascomata solitary ..... *N. globosicarpum*
4b. Ascospores 92–117 × 2–3.8 µm; ascomata in groups of 1–4 ..... *N. nypicola*

5a. Basal appendage keel-like; ascospores 81–107(–126) × 2.5–3.5 µm ... *N. australiense*
5b. Appendage a crescent-shaped or mucilaginous pad 6

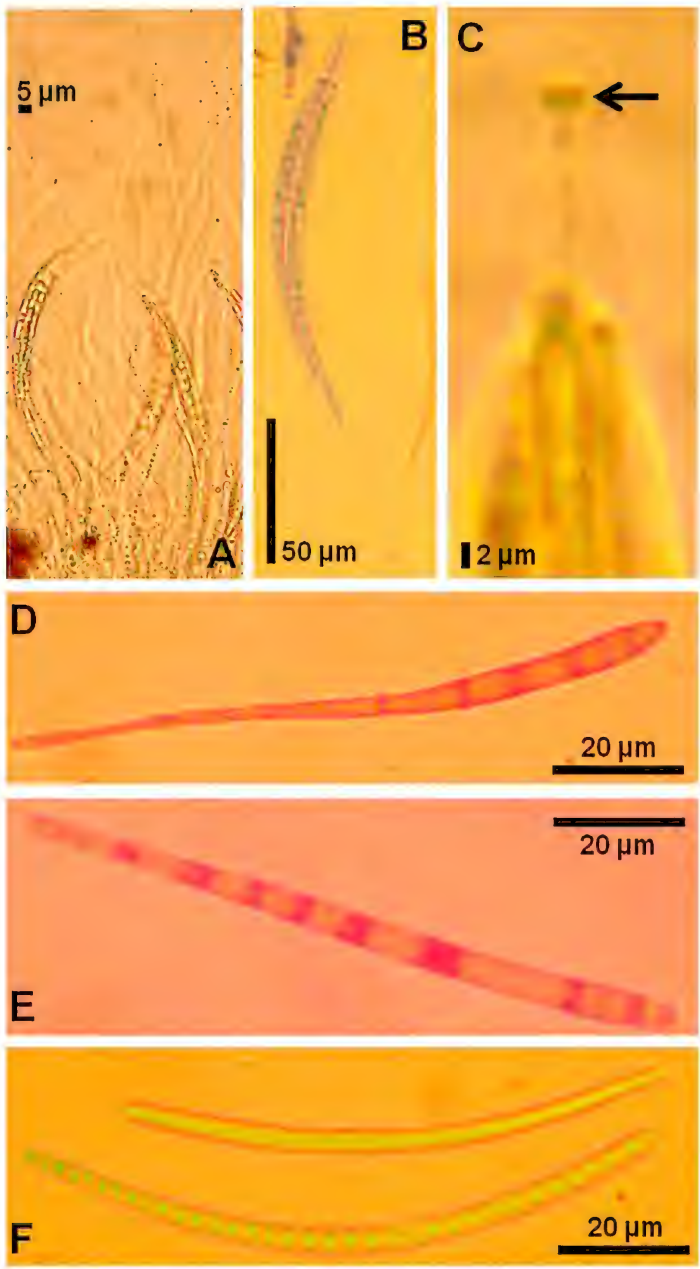

Plate 2. *Neolinocarpon attaleae* (from holotype): A. Paraphyses and asci. B. Ascus. C. Non-amyloid, discoid subapical ring (arrow). D–F. Ascospores.

6a. Crescent-shaped mucilaginous pad at apex; ascospores 68–85 × 2.5–3.5 µm ..... *N. calami*
6b. Mucilaginous appendage at base; ascospores 73–95(–106) × 1.5–2.2 µm ..... *N. eutypoides*

7a. Ascomata inconspicuous; ascospores 76–98 × 2–3 µm ..... *N. inconspicuum*
7b. Ascomata conspicuous 8

8a. On grass (*Poaceae*); ascospores (52.5–)57–64(–84) × 2.5–3 µm ..... *N. penniseti*
8b. On palms (*Arecaceae*) 9

9a. Ascospores (52.5–)57.5–93(–105) × 3–4 µm, filiform-cylindrical, filiform-fusoid to ± clavate ..... *N. attaleae*
9b. Ascospores 114–138 × 2–2.5 µm, filiform, curved ..... *N. nonappendiculatum*

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New *rust* species and hosts from Northern Areas of Pakistan

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Abstract — During a survey of rust fungi of Northern Areas of Pakistan, *Puccinia aegopodii* and *P. rhytismoides* were collected as new to the Indian subcontinent. The uredinial state of *Chrysomyxa pyrolae* is described for the first time from Pakistan on *Pyrola rotundifolia* subsp. *karakoramica,* a new host. *Puccinia swertiae* is reported as a new record for Northern Areas, and *P. oniwaensis* is recorded on *Persicaria orientalis*, a new host.

Key words — Deosai Plains, Fairy Meadows, *Uredinales*

Introduction

Most parts of Northern Areas lie within the watersheds of the Himalaya, Hindu Kush, and Karakoram mountain ranges. Climatic conditions vary widely in the Northern Areas ranging from the monsoon influenced moist temperate zone in the western Himalayas to the semi-arid cold desert in the Northern Karakoram and Hindu Kush. The Northern Areas of Pakistan are well known for their biodiversity. Although these areas contain about 3000 plant species and are floristically rich, they have not been thoroughly surveyed for rust fungi and only approximately 71 species have been reported to date (Ahmed 1956a,b; Gjaerum & Iqbal 1969; Kakishima et al. 1993a,b; Kaneko 1993; Khalid et al. 1995; Khalid & Iqbal 1996, 1997a,b; Ahmed et al. 1997; Sultan et al. 2006a,b, 2008; Iqbal et al. 2008, 2009; Afshan & Khalid 2009; Afshan et al. 2009, 2010, 2011; Ishaq et al. 2011; Khalid & Saba 2011; Saba & Khalid 2011). During our 2010–2011 rust collection trips in the Northern Areas, we discovered *Puccinia aegopodii* and *P. rhytismoides,* both new for the Indian subcontinent and which we report with new hosts and other rust fungi new for these areas of Pakistan.

Materials & methods

Rust fungi were collected from Deosai Plains and Fairy Meadows, Northern Areas, Pakistan. Healthy plants with inflorescences and fruits were collected for accurate

identification. Host plants were identified by comparing them with specimens in the herbarium of the Department of Botany, University of the Punjab, Lahore (LAH).

Free hand sections of infected portions of material and spores were mounted in lactophenol. Semi-permanent slides were prepared by cementing cover slips with nail lacquer (Dade & Gunnell 1969). Preparations were observed under a NIKON YS 100 microscope. Drawings of spores were made by using a Camera Lucida (Ernst Leitz, Wetzlar, Germany). Spores were measured using an ocular micrometer (Zeiss, St Albans, Hertfordshire, England). At least thirty spores were measured for each spore stage. Measurements include the usual range and the arithmetic means; extremes are given in parentheses.

Taxonomy

Chrysomyxa pyrolae (DC.) Rostr., Bot. Centralbl. 5: 127 (1881). FIG. 1

SPERMOGONIA, AECIA and TELIA not seen. UREDINIA scattered evenly on abaxial surface of leaves, *Caeoma*-like with delicate, one-layered peridium, orange-yellow, round, surrounded by ruptured peridium. UREDINIOSPORES globose, obovoid, ellipsoid to polyhedral, hyaline to subhyaline, sometimes with yellowish contents, produced in chains, (15–)18–22 × (18–)22–29 µm (mean 19.8 × 24.7 µm); wall hyaline, 1–2 µm thick, densely verrucose.

MATERIAL EXAMINED: **PAKISTAN**, **NORTHERN AREAS**, Fairy Meadows, on leaves of *Pyrola rotundifolia* subsp. *karakoramica* (Křísa) Y.J. Nasir (*Ericaceae*), with II stage, 23 Jul 2010, coll. & det. Malka Saba 12 (LAH).

COMMENTS: The aecial stage of *Chrysomyxa pyrolae* was reported on cones of *Picea smithiana* (Wall.) Boiss. from Fairy Meadows by Kaneko (1993) and Ahmad et al. (1997). Its uredinial stage from the same locality is newly recorded for Pakistan, and *Pyrola rotundifolia* subsp. *karakoramica* is a new host for this rust fungus.

Puccinia aegopodii (Schumach.) H. Mart., Prodr. Fl. Mosq., Ed. 2: 226 (1817). FIG. 2

SPERMOGONIA, AECIA and UREDINIA not seen. TELIA amphigenous, predominantly hypophyllous, minute, rounded, aggregated, surrounded by ruptured epidermis, pulverulent, black. TELIOSPORES ellipsoid or oblong, cinnamon brown to dark brown, not or slightly constricted at septum, rounded at both ends, sometimes attenuated below, (12–)16–22 × (22–)26–35 µm (mean 18.7 × 30.3 µm); wall dark brown to chestnut brown, 1–2(–2.5) µm, germ pores apical, sometimes subapical or near septum in distal cell, adjacent to septum in proximal cell, mostly with hyaline dome-shaped, up to 3 µm high papilla; pedicel hyaline, short, deciduous, 5–6 × 2–9 µm.

MATERIAL EXAMINED: **PAKISTAN**, **NORTHERN AREAS**, Fairy Meadows, on leaves of *Aegopodium alpestre* Ledeb. (*Apiaceae*), with III stage, 23 Jul 2010, coll. & det. Malka Saba 13 (LAH). **GERMANY**, **BADEN-WÜRTTEMBERG**, Donautal, Inzigkofen, am Strässchen zum Nickhof, kurz vor dem Nickhof, on leaves of *Aegopodium podagraria* L., 16 May 2010, leg. O. Holdenrieder and R. Berndt (ZT Myc 3027).

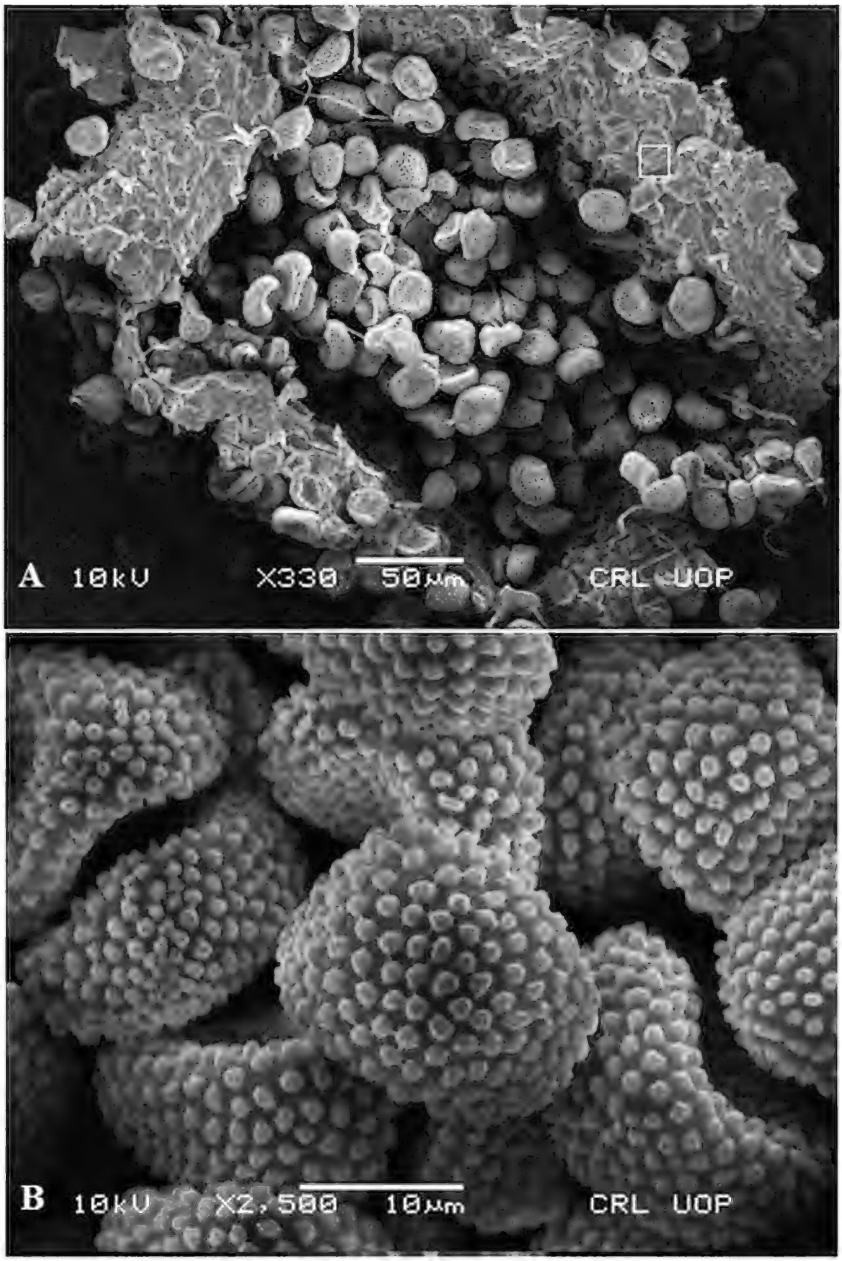

Fig. 1. *Chrysomyxa pyrolae* (SEM). (A) Uredinium with urediniospores and one-layered peridium (square). (B) Urediniospores with densely verrucose ornamentation.

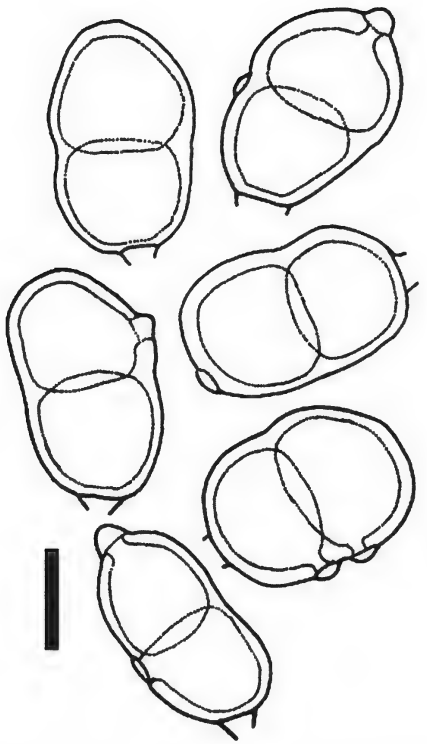

Fig. 2. *Puccinia aegopodii*. Lucida drawings showing position of germ pores on teliospores. Scale bar =15 μm.

Comments: Farr & Rossman 2011 have reported *P. aegopodii* from China and Mongolia on *Aegopodium alpestre*. Our report is an addition to the mycobiota of the Indian subcontinent.

Puccinia oniwaensis Hirats. f. & S. Sato, Trans. Mycol. Soc. Japan 3: 56 (1962). Fig. 3

Spermogonia and Aecia not seen. Uredinia hypophyllous, pulverulent, brown, intermixed with telia. Urediniospores subglobose, ellipsoid to obovoid, pale brown to brown, 15–20 × 22–27 μm; wall 1.2–2 μm, minutely echinulate, germ pores 2, supraequatorial. Telia hypophyllous, sometimes aggregated, pulverulent, black. Teliospores ellipsoid to oblong, rounded at both ends, chestnut brown, slightly or not constricted at septa, 20–22 × 27–34 μm; wall chestnut brown, verrucose, more dense in upper cell; 2–3 μm; germ pore apical in upper cell and near pedicel in lower cell; apex 3–4.5 μm, brown. One and three celled teliospores rarely present.

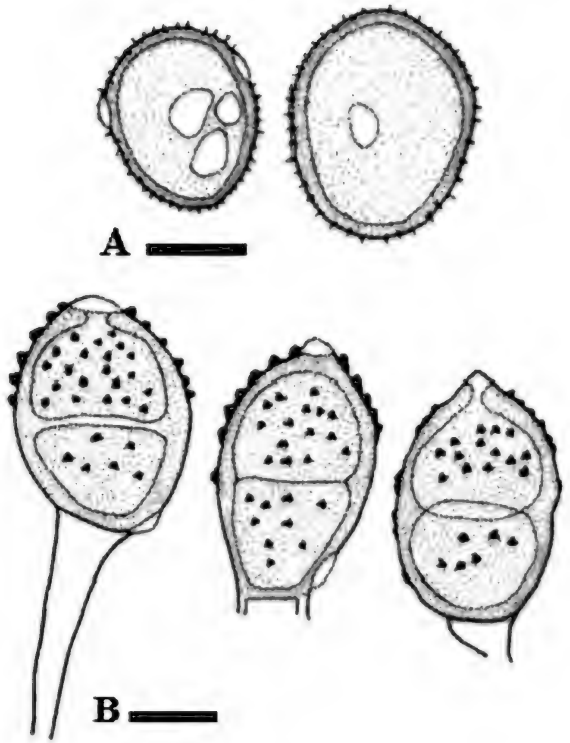

FIG. 3. *Puccinia oniwaensis* (Lucida drawings).
(A) Urediniospores showing minutely echinulate ornamentation.
(B) Teliospores with densely verrucose ornamentation. Scale bar =10 μm.

MATERIAL EXAMINED: **PAKISTAN**, **NORTHERN AREAS**, Deosai Plains, 3300 m a.s.l., on leaves of *Persicaria orientalis* (L.) Spach (*Polygonaceae*), with II + III stages, 10 Sep 2011, coll. Abdul Nasir Khalid, det. Malka Saba 15 (LAH).

COMMENTS: *Puccinia oniwaensis* has been reported on *Polygonum sibiricum* Laxm. from Astore, Rama Lake (Baltistan, Northern Areas) by Khalid et al. (1995). *Persicaria orientalis* is a new host and *P. oniwaensis* is a new report for Deosai Plains.

Puccinia rhytismoides Johanson, Bot. Not.: 173 (1886). FIG. 4

SPERMOGONIA, AECIA and UREDINIA not seen. TELIA hypophyllous, compact, subepidermal, loculate, surrounded by cylindrical paraphyses, 60–80 × 100–200 μm. TELIOSPORES clavate, ellipsoid or oblong, 10–14 × 26–58 μm (mean 11.8 × 40.3 μm), pale brown or brown at apex, cinnamon brown at base, constricted at septum, lower cell longer than apical cell, attenuated at base;

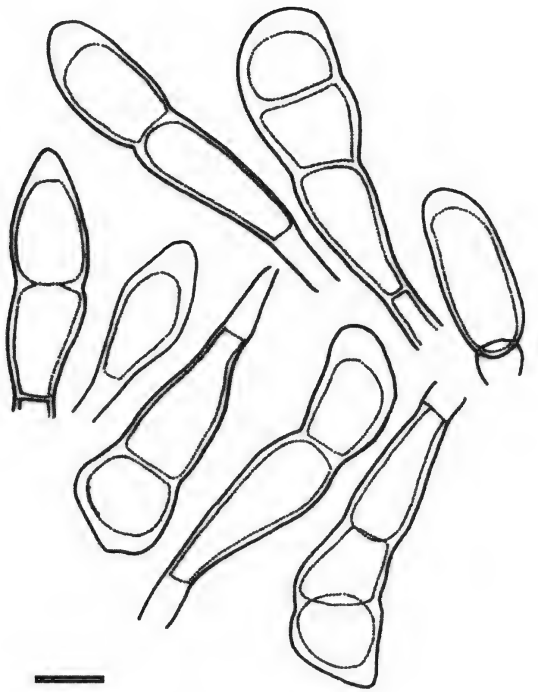

FIG. 4. *Puccinia rhytismoides.* Lucida drawings showing 1–3 celled teliospores. Scale bar =15 μm.

wall smooth, hyaline to pale brown, 0.5–1 μm thick; apex conical, truncated or rounded, 3–6 μm thick; pedicel dark brown, persistent, 4–7 × 3–13 μm. Mesospores common, pale brown, clavate or oblong, 8–10 × 18–30 μm; wall up to 1 μm thick, smooth; apex conical or rounded 4–6 μm; pedicel short, dark brown. Three-celled teliospores rare.

MATERIAL EXAMINED: **PAKISTAN**, **NORTHERN AREAS**, Deosai Plains, on leaves of *Thalictrum alpinum* L. (*Ranunculaceae*), with III stage, 10 Sep 2011, coll. Abdul Nasir Khalid, det. Malka Saba 16 (LAH). **FINLAND, KILPISJÄRVI**, on leaves of *T. alpinum*, 12 Jul 1935, coll. & det. J.I. Liro (ZT Myc s. n.). **NORWAY**, **TRØNDELAG**, near Kongsvold, Doorefjeld, on leaves of *T. alpinum*, 1925, leg. A.G. Eliasson (ZT Myc s.n.).

COMMENTS: *Puccinia rhytismoides,* which represents a new record for the Indian subcontinent, has previously been reported on *Thalictrum alpinum* from Alaska, Finland, Iceland, Norway, Russia, and Sweden and on *T. alpinum* var. *stipitatum* Y. Yabe and *T. tuberiferum* Maxim. in Japan (Farr & Rossman 2011). However Hiratsuka et al. (1992) placed *P. rhytismoides* in synonymy under *P. ustalis* Berk., which Ahmad et al. (1997) from Pakistan reported on *Anemone obtusiloba* D. Don.

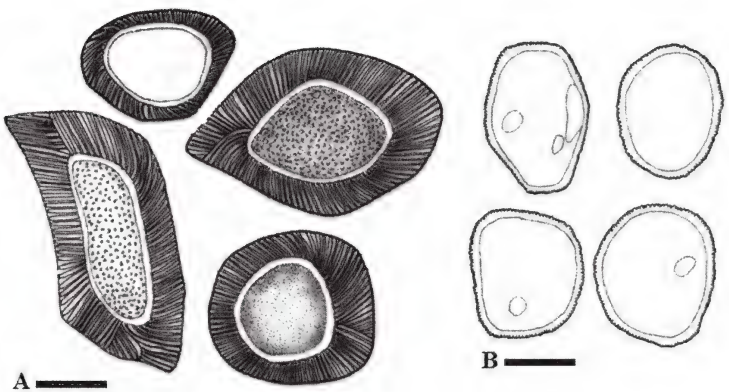

Fig. 5. *Puccinia swertiae* (Lucida drawings).
(A) Peridial cells showing densely verrucose ornamentation.
(B) Aeciospores with minutely verrucose ornamentation. Scale bar =10 μm.

Puccinia swertiae (Opiz) G. Winter, Rabenh. Krypt.-Fl., Ed. 2, 1(1): 205 (1881) ["1884"]. Fig. 5

Spermogonia and Uredinia not seen. Aecia on abaxial side, cupulate, yellowish, gregarious, 200–300 × 250–450 μm. Peridial cells yellowish brown, rhomboidal to oblong or irregular, 17.7–39 × 22–53 μm (mean 35.3–37.9 μm); wall densely verrucose, 2.8–8.5 μm. Aeciospores obovoid or subglobose, hyaline to brown, 19.6–25.2 × 20.5–27(–36) μm (mean 21.8 × 24.4 μm); wall finely verrucose, 0.5–1.2 μm. Telia abaxial, black, pulverulent, covered by ruptured epidermis, scattered, naked, 100–200 × 200–800 μm. Teliospores obovoid or ellipsoid, rounded at both ends, not or hardly constricted at septum, dark brown to chestnut brown, 25–30.4 × 31–39 μm (mean 27 × 35 μm); wall verrucose, 1.8–3.5(–4) μm; germ pores two, next to septum in both upper and lower cells, rarely apical in upper cell; apex dark brown to chestnut brown, not or slightly thickened, 1.2–2 μm; pedicel short, hyaline, persistent, 4–9.4 × 2.6–8.5 μm.

Material examined: **PAKISTAN**, **Northern Areas**, Deosai Plains, on leaves of *Swertia petiolata* D. Don (*Gentianaceae*), with I + III stages, 10 Sep 2011, coll. Abdul Nasir Khalid, det. Malka Saba 17 (LAH).

Comments: *Puccinia swertiae* has been reported on *Swertia petiolata* and *S. speciosa* Wall. from Kalam (Swat, Khyber Pakhtunkhwa), Sargodha, and Shogran (Kaghan Valley, Khyber Pakhtunkhwa) (Ahmad 1956a,b; Jorstad & Iqbal 1967; Ono & Kakishima 1992; Ono 1992). *Puccinia swertiae* represents a new record for Deosai Plains.

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New records of lichens from Chile

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Abstract — After fieldwork in central Chile, five lichen species were established as new for the country: *Bryoria lanestris*, *Buellia disciformis*, *Haematomma sorediatum*, *Lecidea capensis*, and *Punctelia rudecta*. They were discovered in two national parks in the Maule Region and the area surrounding the Termas de Chillán, Bío-Bío Region. Each species is briefly described, with habit illustrations and comments on chemistry, habitat, and morphology. These collections suggest a high potential for finding more unreported lichen species in Chile's national parks.

Key words — lichenized fungi, South America, floristic survey, taxonomy

Introduction

In Chile, the climate varies from arid desert in the north, through humid subtropical in Easter Island, to near polar in the south, extending across 38 degrees of latitude. The five species reported here were collected in two national parks with a Mediterranean climate in the central part of Chile. About 1400 lichen species have been previously reported from Chile (Galloway & Quilhot 1998). Our new findings cover only a very small part of the country, but indicate that as it has such a wide range of different climates, many more species may still be discovered, and the total number of lichen species occurring in Chile is likely to be far higher than currently reported.

Materials & methods

The 405 specimens sampled during the current collections are deposited in the Korean Lichen Research Institute, Sunchon National University, South Korea (KoLRI).

The specimens were examined by standard microscopical techniques, hand-sectioned under a Nikon SMZ 645 dissecting microscope. Anatomical descriptions were based on observations under a Nikon Eclipse E200 microscope. Secondary metabolites were identified by TLC according to Elix et al. (1987), Orange et al. (2001), and White & James (1985). Medullas were tested with IKI (10% aqueous potassium iodide) for identification.

Results

Bryoria lanestris (Ach.) Brodo & D. Hawksw. FIG. 1A, B

Thallus fruticose to subpendent, attached to the substrate by one holdfast, surface pale olive brown to pale brown, smooth, 0.2–0.4 mm thick, 1–5 cm long, main branch usually isotomic, with few side branches, surface with white soralia, growing from the fissures, without pseudocyphellae.

CHEMISTRY: Thallus P+ red or P–, medulla P–, soralia C–, K–, P+ red, containing fumarprotocetraric acid (by TLC).

HABITAT: On the bark of *Nothofagus* sp. Mainly distributed in the northern boreal zone (Brodo & Hawksworth 1977).

SPECIMEN EXAMINED: **CHILE**, MAULE REGION, TALCA PROVINCE, National Reserve "Altos de Lircay", 35°36′08.2″S 71°02′56.7″W, alt. 1367 m a.s.l., on bark, 26 January 2012, J.-S. Hur CL-120099-1 (KoLRI 014265).

REMARKS: The only *Bryoria* species previously reported in Chile is *B. chalybeiformis* (Galloway & Quilhot 1998), which grows on rock or soil, without soralia but with spinulose branches, its color is much darker, and it is shiny. *Bryoria simplicior,* which might also be confused with *B. lanestris,* can be distinguished by its roundish and greenish black soralia that are broader than the branch and by its lack of compounds.

Buellia disciformis (Fr.) Mudd FIG. 1C, D

Thallus crustose, thin and rimose, immersed or superficial, surface pale white to whitish green, prothallus present when meeting with other thalli, black. Apothecia lecideine, disc black and plane, without pruina, with smooth margin, 0.2–0.4 mm in diam., spores 8 per ascus, brown, 1-septate, 14–21 × 6–9 μm.

CHEMISTRY: Thallus K+ yellow, containing atranorin (by TLC).

HABITAT: On bark, mainly distributed in temperate areas (Bungartz et al. 2007)

SPECIMEN EXAMINED: **CHILE**, MAULE REGION, CAUQUENES PROVINCE, National Reserve "Los Ruiles", 35°49′49.3″S 72°30′25.5″W, alt. 342 m a.s.l., on bark, 28 January 2012, J.-S. Hur CL-120240 (KoLRI 014407).

REMARKS: *Buellia disciformis* might be confused with some *Lecidella* species, which also grow on bark and are morphologically quite similar, but it can be distinguished by its brown septate spores and K+ yellow reaction (atranorin).

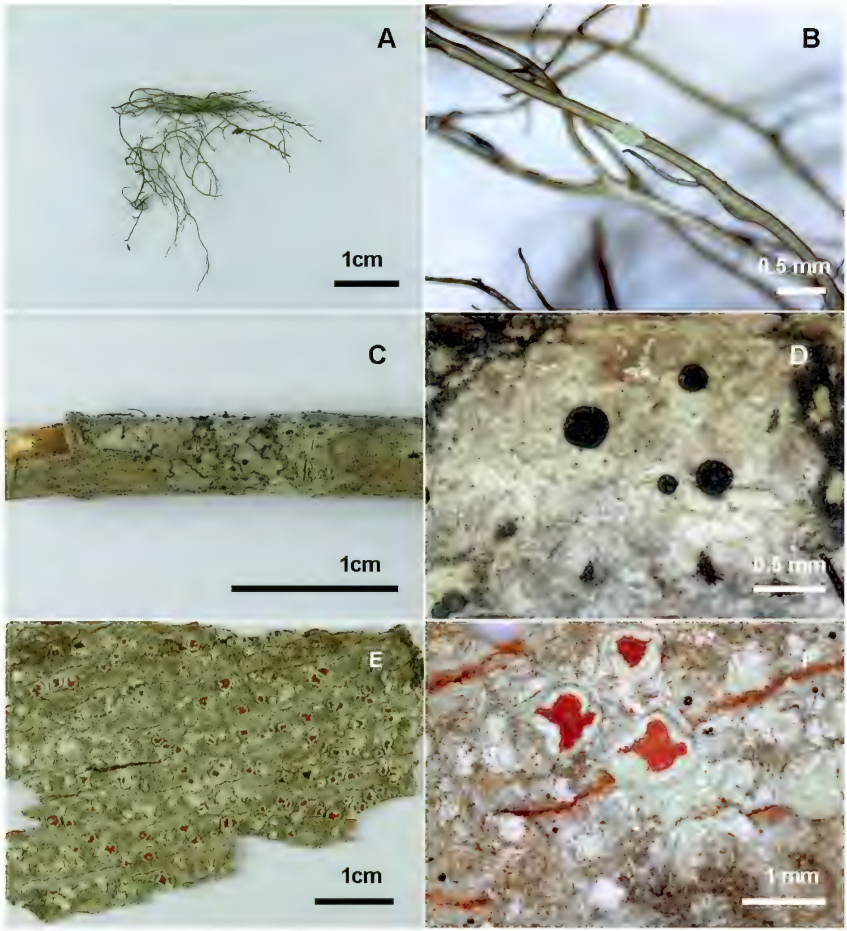

FIG. 1 Habit of lichens new to Chile. A–B. *Bryoria lanestris*. C–D. *Buellia disciformis*. E–F. *Haematomma sorediatum*.

Buellia chloroleuca could be easily confused with *B. disciformis*, but its distinctly C+ orange (xanthone) reaction readily distinguishes it.

Haematomma sorediatum R.W. Rogers FIG. 1E, F

Thallus crustose, white to greenish gray, granulose, soralia present, scattered on the surface of the thallus or on the margin of apothecia, orbicular, white. Apothecia lecanorine, up to 1 mm in diam., disc bright red, flat, epruinose, margin persistent, crenulate, spores 8 per ascus, hyaline, 7–12 septate, straight fusiform, 40–70 × 5–7 µm.

Chemistry: Thallus and soralia K+ yellow, C–, KC+ yellow, P+ pale yellow, containing atranorin and placodialic acid (by TLC).

Habitat: On bark. It has cosmopolitan distribution (Brodo et al. 2008).

Specimen examined: **CHILE**, Maule Region, Cauquenes Province, National Reserve "Los Ruiles", 35°50′02.4″S 72°′30′35.3″W, alt. 175 m a.s.l., 28 January 2012, J.-S. Hur CL-120158 (KoLRI 014324).

Remarks: The only *Haematomma* species from Chile can easily be separated from other species by its roundish soralia on the apothecial surface and margin and a red apothecial disc that is sometimes covered with pruina. Staiger & Kalb (1995) treated *H. neglectum* as a synonym of *H. sorediatum*, but Brodo et al. (2008) still had doubts about this. As the two species need more study, we use the name *H. sorediatum* here in the strict sense.

Lecidea capensis Zahlbr. Fig. 2A, B

Thallus squamulose to areolate, reddish brown to brown, smooth, convex, usually shiny, marginal part white, prothallus black, medulla I–. Apothecia black, round and subimmersed, pruinose, 0.5–1.3 mm in diam., hymenium 40–60 µm high, hyaline, epihymenium greenish black, spores ellipsoid, 10–14 × 3–4 µm.

Chemistry: Thallus and medulla all negative in spot reaction, containing 2'-O-methylperlatolic acid (by TLC).

Habitat: On rock, known from coastal forest and South Africa and Australia (Galloway 2007).

Specimen examined: **CHILE**, **Bío-Bío Region**, Chillán Province, vicinity of Termas de Chillán, 36°54′21.6″S 71°24′05.5″W, alt. 1980 m a.s.l., 3 February 2012, J.-S. Hur CL-120360 (KoLRI 014529).

Remarks: The species is characterized by saxicolous habit and a convex shiny thallus that is areolate and pale brown to reddish brown. It is similar to *L. fuscoatrula*, which differs in its I+ violet medulla reaction, smaller (<10 µm) ascospores, dark brown hymenium, and 2'-O-methylmicrophyllinic acid as the main compound.

Punctelia rudecta (Ach.) Krog Fig. 2C, D

Thallus foliose, greenish gray, lobes plane and linear, 3–7 mm wide, covered with white pseudocyphellae, irregular in shape, isidia mostly on the central part of the thallus, simple or branched, sometimes lobule-like, brown-tipped, lower surface pale brown, with light brown rhizines, simple or forked. Apothecia not seen.

Chemistry: Thallus K+ yellow, C–, medulla K–, C+ red, containing atranorin and lecanoric acid (by TLC).

Habitat: On rock; common in North America, Europe, Australia, and Asia (Egan & Aptroot, 2004)

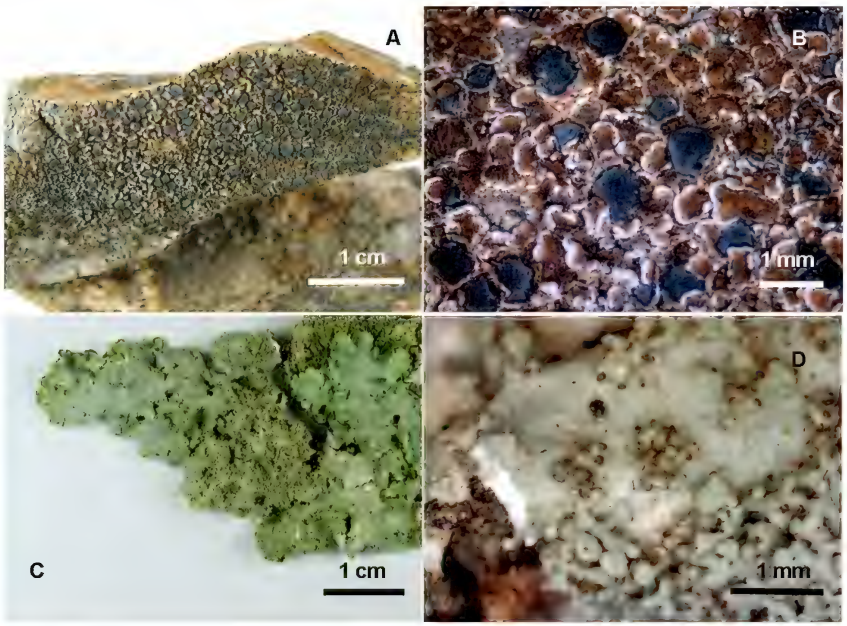

Fig. 2 Habit of lichens new to Chile. A–B. *Lecidea capensis*. C–D. *Punctelia rudecta*.

Specimen examined: **CHILE**, Maule Region, Cauquenes Province, National Reserve "Los Ruiles", 35°50′02.4″S 72°30′35.3″W, alt. 175 m a.s.l., 28 January 2012, J.-S. Hur CL-120142 (KoLRI 014308).

Remarks: This is the only *Punctelia* species with isidia reported from Chile. It is similar to *P. borreri*, which has soredia on the lobe margins instead of isidia. As all other *Punctelia* species reported in Chile before have soralia, they can easily be separated from *P. rudecta*. It is common and widespread in eastern and southeastern North America (Brodo et al. 2001).

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Endocarpon maritima sp. nov. (lichenized *Ascomycota*) from the maritime region of South Korea

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Abstract — A new lichenized fungus, *Endocarpon maritima*, was collected from the rocky seashores of Bogil Island, South Korea. The species is characterized by a squamulose to lobate brown to olive-green thallus attached to the substratum by rhizohyphae, blackish hypothallus, 2-spored asci, spores 25–37.5(–50) × (10.5–)13–17(–20) µm, and a maritime distribution. Lichenized species of *Aspicilia, Buellia, Caloplaca, Phaeophyscia, Physcia,* and *Pterygiopsis* occur in close association with *E. maritima*.

Key words — pyrenocarpous lichen, taxonomy, *Verrucariaceae*

Introduction

The cosmopolitan pyrenocarpous squamulose to subfoliose lichen genus *Endocarpon* Hedw. is represented by ca. 50 species (Kirk et al. 2008) and occupies a wide range of habitats, such as terrestrial, maritime, or fresh water, depending on the species. The genus has been well worked out in India (Singh & Upreti 1984), Australia (McCarthy 1991), New Zealand (Galloway 2007), Japan (Harada 1993), Mexico (Breuss 2000), the Greater Sonoran Desert (Breuss 2002), Costa Rica (Aptroot et al. 2008), and Great Britain (Orange 2008, Giavarini & Fox 2009). However, the genus is not well researched from South Korea and so far is represented by only 4 species (Joshi et al. 2009, Moon & Aptroot 2009).

The maritime *Endocarpon* species comprise important elements in the intertidal and splash zones at the boundary of terrestrial biota, frequently forming with *Verrucaria* a blackish zone. Extensive field surveys of the maritime regions conducted by one author (YJ) during his stay in South Korea revealed an *Endocarpon* species new to science, described here.

Materials & methods

Studied specimens are deposited in KoLRI (Korean Lichen Research Institute, Sunchon, South Korea) and CBM (Natural History Museum and Institute, Chiba, Japan). Description and photographs of external morphology are based on air-dried material observed under a dissecting stereomicroscope (Nikon SMZ 645). Sections made by a razor blade under the stereomicroscope were mounted in lactophenol cotton blue. Anatomical descriptions are based on these preparations studied by a compound microscope (Nikon Eclipse E200). Measurements of ascospores were made at ×400 magnification mounted in water; only free ascospores lying outside the asci were measured. Secondary metabolites were identified by TLC as described by White & James (1985) and Orange et al. (2001) using solvent system C. The mycological terminology follows Kirk et al. (2008).

New species

Endocarpon maritima Y. Joshi & Hur, **sp. nov.** FIG. 1

MycoBank MB 801275

Similis Endocarpon ramulosum, E. neopallidulum *et* E. japonicum, *sed differt in maritimum habitio und hypothallus praesens.*

Type: South Korea. Jeollanam-do: Bogil-myeon, Bogil Island, Tong-ri, near Tongri Beach, 34°09′68.4″N 126°35′15.3″E, alt. ca 3 m a.s.l., on exposed rocks, 6 February 2010, Y. Joshi, H. S. Jeon, M. H. Jeong 100197 (**holotype**, KoLRI; **isotype** CBM).

Etymology — The specific epithet refers to its occurrence in the maritime region of South Korea.

Thallus squamulose to lobate, brownish to olive-green, 120–130 µm thick, composed of loosely aggregated squamules, squamules usually lobate at maturity; hypothallus ±present, black, extending beyond the squamules or lobes. Squamules usually adnate or apparently ascending near the margin. Lobules 0.2–0.6 × 0.2–0.4 mm, sparsely or repeatedly, irregularly branched, frequently imbricate, discrete to ±contiguous, margins entire, ±recurved upwards at margin; shape of lobules regular; apically rounded, ±darker than rest of the thallus. Upper surface brown to ±pale brown with olive tinge, slightly glossy, smooth, flat to slightly convex. Lower surface almost black around attaching parts, with sparse rhizohyphae, lacking rhizines. Squamules attached to the substratum at basal ends or central part. Upper cortex paraplectenchymatous, 12.5–20 µm thick, ±pale brown in the uppermost part, hyaline in the remainder. Medulla white, composed of spherical hyphal cells, 20–35 µm thick. Algal layer 50 µm thick, photobiont solitary, in vertical rows in the upper part of layer. Lower cortex undifferentiated from the medulla, dark brown to almost black, 25 µm thick.

Perithecia numerous, 1–4 per squamule/lobule, black, laminal, immersed, globose to somewhat depressed, generally a small black area around ostiole is emerging like a prominent protuberance, ostioles like pale to dark dots or

FIGURE 1: *Endocarpon maritima* (habit). Scale bar = 5 mm.

indistinct, perithecia pyriform in cross sections, (220–)230 × 250(–270) µm. EXCIPLE dark brown to almost black, 50 µm thick. SUBHYMENIUM 25 µm thick. PERIPHYSES 20–50 µm long. HYMENIUM 70–87.5 × 187.5–237.5 µm, hymenial algae spherical, 5–7.5 µm across. ASCI clavate, 75–100 × 20–25 µm, 2-spored. ASCOSPORES hyaline to very pale brown, muriform, ellipsoidal to bacilliform, 25–37.5(–50) × (10.5–)13–17(–20) µm. PYCNIDIA and conidia not seen.

CHEMISTRY — Spot test reactions: thallus K–, C–, P–. TLC: no lichen substances detected.

ECOLOGY & DISTRIBUTION — The new taxon is thus far confined to the maritime region of South Korea, where it grows luxuriantly over non-calcareous (limestone) rocks in close association with *Aspicilia caesiocinerea* (Nyl. ex Malbr.) Arnold, *Buellia badia* (Fr.) A. Massal., *Caloplaca bogilana* Y. Joshi & Hur, *C. cinnabarina* (Ach.) Zahlbr., *Phaeophyscia hirtuosa* (Kremp.) Essl., *Physcia stellaris* (L.) Nyl., and *Pterygiopsis affinis* (A. Massal.) Henssen. This is a second new species from Bogil Island (the other being *Caloplaca bogilana*) and is so far known only from this island.

REMARKS — The new taxon is characterized by a squamulose to lobate, brown to olive-green thallus attached to substratum via rhizohyphae, ±blackish hypothallus, 2-spored asci, and maritime distribution. *Endocarpon ramulosum*

H. Harada and *E. neopallidulum* H. Harada from Japan differ in lacking a hypothallus and being strictly inland species growing between elevations of 200–900 m and 100–150 m, respectively. *Endocarpon japonicum* H. Harada, another closely related species, differs in its different substrate specificity (growth at the edge of streams on partially submerged rocks), absence of a hypothallus, and its inland distribution between elevations of 50–700 m. In its lobate form, *E. petrolepideum* (Nyl.) Nyl. would not be confused with *E. maritima,* because its squamules are mono-lobed and generally scattered to adjacent but not overlapping. *Endocarpon nigromarginatum* H. Harada resembles *E. maritima* in having squamules with conspicuous black borders (i.e. margins) but differs in having both rhizines and rhizohyphae on the lower surface and a ±pachydermatous upper cortex. The presence of a black hypothallus confuses the new taxon with *Endocarpon nigrozonatum* Ajay Singh & Upreti, which differs in having an inland distribution and strictly squamulose morphology.

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Two striking *Inocybe* species from Yunnan Province, China

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Abstract —Two striking species, *Inocybe caroticolor* sp. nov. and *I. olivaceonigra,* are documented in this paper. *Inocybe caroticolor* is characterized by its carrot-colored basidiomata, entirely pruinose stipe, aromatic odor, and nodulose basidiospores. *Inocybe olivaceonigra*, originally described from Papua New Guinea, is new to China. Illustrated descriptions of the two species are provided based on the Chinese collections. DNA sequences of internal transcribed spacer regions (ITS) of the Chinese materials were generated, analyzed, and submitted to the GenBank.

Key words — *Agaricales, Inocybaceae,* taxonomy

Introduction

Inocybe (Fr.) Fr. is a large agaric genus, which was established as a "tribe" of *Agaricus* by Fries in 1821 and elevated to genus rank in 1863. Currently, there are about 500 (Kirk et al. 2008) to 700 (Matheny et al. 2009) species of *Inocybe* recognized worldwide. In the last few years, new species of *Inocybe* continue to be described from around the world (Matheny & Bougher 2005, Kobayashi 2005, 2009; Jacobsson & Larsson 2009, Vauras & Kokkonen 2009, Kobayashi & Onishi 2010, Kropp & Albee-Scott 2010, Kropp et al. 2010, Kokkonen & Vauras 2011, Vauras & Larsson 2012).

In East Asia, more than 140 species of *Inocybe* have been recorded in Japan (Kobayashi 2002, 2005, 2009). This number is still increasing (Kobayashi & Onishi 2010). At present, there are ninety-eight names of *Inocybe* reported from China (Fan & Bau 2010). Unfortunately, many of the names recorded in China lack detailed descriptions or information on voucher specimens. Although a few studies on species diversity of *Inocybe* have been undertaken in some provinces (Liu 1987, Zheng et al. 1985), rather few species have been originally described as new from China (Wang 1973). Monographic studies on species diversity of *Inocybe* in China are needed.

During taxonomic studies on *Inocybaceae* from China, we encountered two impressive species easy to recognize by their conspicuous colors in the field.

Inocybe caroticolor is proposed as new based on comparative studies of available collections. Another species is found to be conspecific with *I. olivaceonigra* after examining the holotype. It is re-described based on Chinese collections. DNA sequences of internal transcribed spacer regions (ITS) of the Chinese materials were generated and analyzed.

Materials & methods

Materials were collected in *Castanopsis* forests with a subtropical highland climate at 1900 m elevation in Yunnan Province (25°03′N 102°37′E and 25°08′N 102°44′E), and examined specimens were deposited in the Herbarium of Mycology, Jilin Agricultural University (HMJAU). Additional Chinese collections from KUN (with HKAS numbers) and authentic specimens of *I. lutea* Kobayasi & Hongo from TNS and *I. olivaceonigra* and "*Astrosporina lutea*" from Z+ZT were examined (Herbarium abbreviations follow Thiers (2012)). Macroscopic characters were described based on field notes. Microscopic features were made from slide preparations mounted in 5% KOH solution and Congo Red. Measurements of microscopic features were made at 1000× on an Nikon 80i microscope. Color notations follow Kornerup & Wanscher (1981). Measurement methods follow Kobayashi (2002, 2009).

Genomic DNA was extracted from silica-dried materials with a modified CTAB protocol (Doyle & Doyle 1987). ITS gene fragments were amplified with primers ITS1F and ITS4B (Gardes & Bruns 1993). Sequencing was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd, Shanghai, China). Five new sequences (JX025772–JX025776) have been deposited at GenBank. The new sequences were used as queries of the NCBI nucleotide sequence database using the BLASTn search algorithm.

Taxonomy

Inocybe caroticolor T. Bau & Y.G. Fan **sp. nov.** FIGS 1, 2

MYCOBANK: MB 564971

Differs from *Inocybe lutea* by its hymenial cystidia with thicker walls, its stipe covered uniformly by caulocystidia, and its aromatic odor.

TYPE: China, Yunnan Province, Kunming, Heilongtan Park, under *Quercus variabilis* Blume, 26 Aug 2010, Y.G. Fan 2010126 (**Holotype**, HMJAU 23271; GenBank JX025774).

ETYMOLOGY: Refers to the carrot color of the basidioma.

BASIDIOMA small. PILEUS 17–33 mm in diam, conical-convex when young, then plano-convex, dry, with a prominent obtuse umbo, covered with appressed squamules, radially arranged, fibrillose, rimulose to usually rimose at margin, margin inrolled when young, occasionally recurved when mature, orange (5A7-8) to apricot (4A7-8), squamules concolorous when young, then turning brown (5C8) to reddish brown (6C8), background orange-apricot (4A8) to ochraceous (3A6). LAMELLAE adnexed, moderately crowded, up to 3 mm in width, pale

FIG. 1. *Inocybe caroticolor* basidiomata (HMJAU 24614).

orange to apricot (4A7-8) when young, dirty apricot (4B7) to brown (5B7) when mature, gill edge concolorous or paler, not smooth. STIPE 30–42 × 2–3 mm, solid, pale orange (5A6) to apricot (4A7), equal with subbulbous to non-marginate bulbous base, occasionally base distinctly wider, entirely pruinose on the stipe surface, densely pruinose at upper part, longitudinally striate downwards, stipe base with whitish tomentum. CORTINA absent. CONTEXT with conspicuous aromatic odor, fleshy in pileus, whitish to pale apricot (4A5), 1–3 mm in width, striate in stipe, pale apricot (4A5).

BASIDIOSPORES 6.3–9.3(–9.5) × (4.4–)4.7–6.0(–6.2) μm, Q = (1.43–)1.47–1.58(–1.61), weakly to prominently nodulose (with 7–9 nodules), yellowish brown. BASIDIA 24–37 × 5–9 μm, with 4 sterigmata, occasionally with 1 or 2 sterigmata, clavate, narrower downwards, at times slightly enlarged at the base, usually with yellowish pigments. PLEUROCYSTIDIA 48–63 × 12–16 μm, fusiform with obtuse apices, occasionally utriform, crystalliferous at apex, base obtuse or tapering into pedicel, walls bright yellow, 2–3(–4) μm thick, hyaline inside, rarely with yellow pigments. CHEILOCYSTIDIA similar to pleurocystidia, more variable, occasionally cylindric; PARACYSTIDIA abundant among cheilocystidia, clavate to obovoid, thin-walled, hyaline, mixed with basidia. CAULOCYSTIDIA descending to stipe base, 48–79 × 9–14 μm, abundant, similar to pleurocystidia, but more variable in shape, usually with slender habit and thinner walls (0.8–2.4 μm); CAULOPARACYSTIDIA abundant, thin-walled, fusoid to lageniform, at times apex tapered, mixed with filamentous hyphae. PILEIPELLIS a cutis,

composed of cylindric, smooth hyphae 2.0–7.0 µm wide, regularly arranged, with oil-yellow to dark-yellow intra-hyphal pigments in upper layer, almost hyaline in lower layer. Clamp connections present in all tissues, but not at every septum.

Habitat and ecology: Singly in groups, common in roadsides near forests, all known collections under *Quercus variabilis* except for one (HKAS 38963) under *Pinus yunnanensis*. June to August in Yunnan, China.

Additional specimens examined: ***Inocybe caroticolor*** — **CHINA, Yunnan Prov.**, Kunming: Kunming Botanical Garden, alt. 1900 m, under *Quercus variabilis*, 24 Jul 2011, Y.G. Fan 2011123 (HMJAU 24614; GenBank JX025773); 27 Aug 2010, Y.G. Fan 2010126c (HMJAU 24621); 27 Aug 2010, Y.G. Fan 2010126d (HMJAU 24622); 28 Aug 2010, Y.G. Fan 2010126e (HMJAU 24623); 26 Jun 2000, X.H. Wang 977 (HKAS 36740); Qiongzhu temple, alt. 2100m, under *Quercus variabilis*, 26 Jul 2011 Y.G. Fan 2011139 (HMJAU 24615; GenBank JX025772); Wuding: Shizishan, under *Pinus yunnanensis* Franch., 18 Aug 2000, F.Q. Yu 168 (HKAS 38963).

Inocybe lutea — **JAPAN, Shiga Pref.** Otsu city, Ishiyama-dera, on the ground in forest, 29 Sep 1955, T. Hongo 1285 (TNS-F-237714); **Ishikawa pref.**, Tatsunokuchi Hill Park, under Fagacea forest, 22 Jul 1995, G. Kazuo (TNS-F-32353).

"***Astrosporina lutea***" — **PAPUA NEW GUINEA, Morobe District**, Bulolo, Manki, alt. 1400 m, under *Castanopsis* and *Lithocarpus*, 28 Mar 1972, E. Horak (ZT Myc11121 [= ZT 72/335]).

Comments: *Inocybe caroticolor* can be easily recognized in the field by its carrot color, distinct aromatic odor, and entirely pruinose stipe. Microscopically, the basidiospores are nodulose, hymenial cystidia are thick-walled, and caulocystidia descend to the base of stipe. The basidiospore outline is variable. Certain collections or individuals predominately possess weakly nodulose basidiospores (HMJAU 24615, and one basidioma from HMJAU 24614, as in Fig. 1c). However, some collections or individual basidiomata possess predominately strongly nodulose basidiospores with 7–9 nodules (as in the remaining basidiomata of HMJAU 24614, HKAS 38963, and HKAS 36740; see Fig. 1e). An intermediate phenotype also exists (HMJAU 23271, HMJAU 24621, HMJAU 24622 and HMJAU 24623; see Fig. 1d). However, the ITS sequences from HMJAU 24615: GenBank JX025772 and the strongly nodulose-spored material of HMJAU 24614: JX025773 are identical with that from the holotype (HMJAU 23271: JX025774) except for a single base difference in HMJAU 24614. Furthermore, no distinct morphological difference was found among these collections. We consequently interpret the basidiospore shape divergence as intraspecific variation.

Few nodulose-spored *Inocybe* species resemble *I. caroticolor*. *Inocybe bresadolae* Massee has a similar basidiospore shape and sweet smell, but its pileus and stipe have only an indistinct orange tint that is found only in older specimens (Hobart & Tortelli 2009). Furthermore, *I. bresadolae* has a more robust habit and a context that reddens after cutting (Massee 1904, Horak

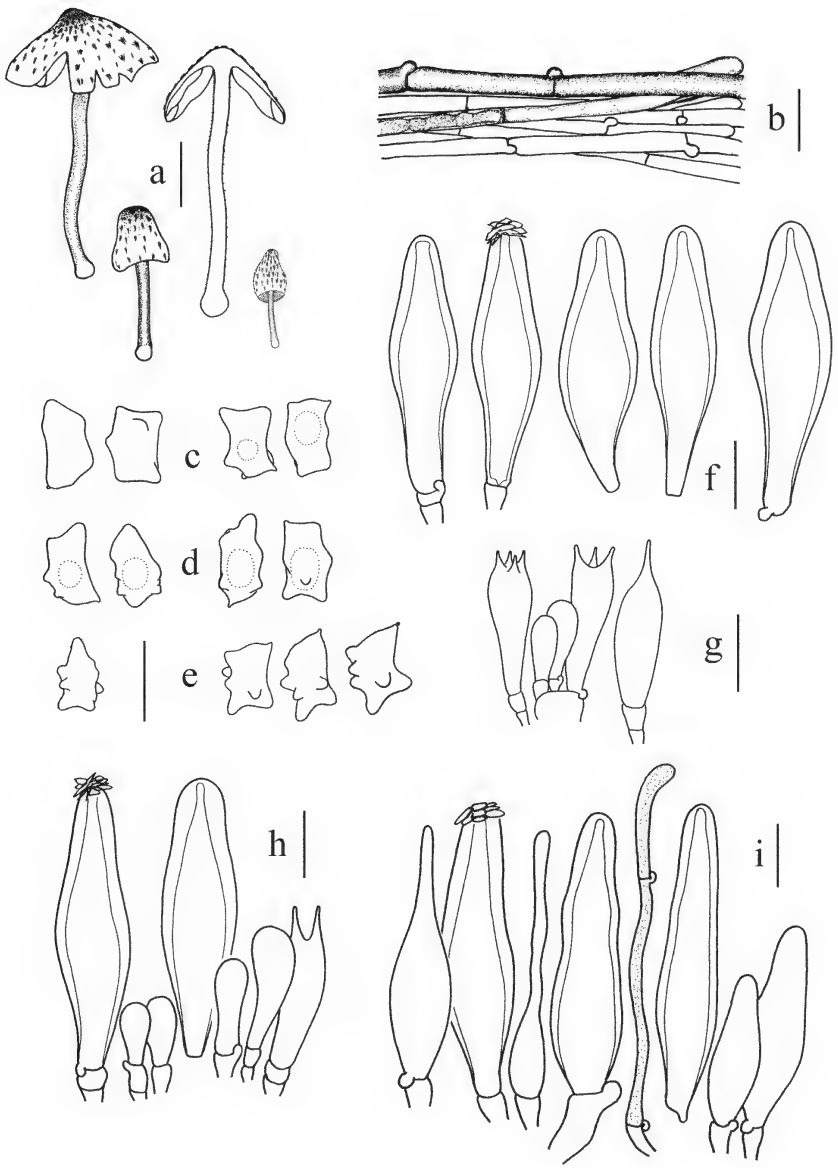

Fig. 2. *Inocybe caroticolor* (HMJAU 23271, holotype [except for c & e]). a. Basidiomata. b. Pileipellis. c. Basidiospores (HMJAU 24615). d. Basidiospores. e. Basidiospores (HMJAU 24614). f. Pleurocystidia. g. Basidia and sterile cells. h. Cheilocystidia, paracystidia, and basidium. i. Caulocystidia, paracystidia, and hypha. Scale bars: a = 10 mm, b–e = 10 μm, f–i = 20 μm.

1979). An East Asian taxon, *I. umbratica* f. *aurantiaca* Takah. Kobay., which resembles the new species to some extent, has a marginate stipe base, fungoid or grassy to spermatic odor, and its type was collected from a *Abies mariesii*, *Pinus koraiensis*, and *Betula ermanii* forest (Kobayashi 2002). *Inocybe polycystidiata* Kobayasi, described from Japan, also resembles the new species but differs by its subviscid pileus (Kobayasi 1952), recurved pileus scales (Kobayashi 2002), shorter basidia, and smaller basidiospores (Kobayasi 1952 = 5–6.5 × 4.5–5 μm; Kobayashi 2002 = 5.4–7.3 × 4.2–5.9 μm, Q = 1.1–1.5).

Another East Asian species, *I. lutea,* also resembles the new species. We have examined the authentic materials of *I. lutea* identified by Hongo (TNS-F-32353, TNS-F-237714), who helped describe the species. Our examinations reveal that it can be distinguished by the following features: (1) the pileipellis hyphae are hyaline with only a slight yellow tinge; (2) the hymenial cystidia are broadly fusiform and slightly thick-walled (1–2 μm thick); and (3) caulocystidia are present only at the stipe apex. An examination of the syntype and additional collections of *I. lutea* also reveal that caulocystidia are restricted to the stipe apex (Kobayashi 2002). Furthermore, Kobayasi (1952) originally described *I. lutea* as having a yellow fibrillose stipe that tapers upwards and an "iodine-like" odor.

Lastly, *I. lutea* as described by Horak (1979, as "*Astrosporina lutea*" (Kobayasi & Hongo) E. Horak) based on materials from Papua New Guinea is very close to *I. caroticolor* in basidioma color and entirely pruinose stipe but differs by smaller basidiospores (5.5–8 × 5–6 μm), a burnt horn smell, a marginate stipe base, and a fibrillose pileus with brown fibrils only. Our examinations of a voucher specimen of the Papua New Guinea material (ZT Myc11121) reveal pileipellis hyphae identical to those of *I. caroticolor* and hymenial cystidia that are predominately utriform usually with a truncate and strikingly thickened base. The Papua New Guinea "*Astrosporina lutea*" collections appear to share more similarities with *I. caroticolor* than with East Asian materials of *I. lutea*. However, "*A. lutea*" differs from both sets of materials at least by its unique odor.

BLASTn results of our *I. caroticolor* ITS sequences show one match (95% similarity) with unidentified environmental sequence (Wilson et al. 2008). Also, one sequence by Ryberg et al. (2008) from Estonian material labeled *I.* aff. *grammata* reaches 95% similarity.

Inocybe olivaceonigra (E. Horak) Garrido, Biblioth. Mycol. 120: 177, 1988. Figs 3–6
≡ *Astrosporina olivaceonigra* E. Horak, Persoonia 10(2): 194, 1979.

Basidiomata small, slender. Pileus 11–23 mm diam., conical at first, then convex to applanate with a blunt umbo, usually rimose or occasionally uplifted or recurved at margin in age, mostly silky-smooth, rarely scaly,

FIG. 3. *Inocybe olivaceonigra* basidiomata (HMJAU 24616).

entirely deep green (27A7-8) at first, then fading to olivaceous (28b7-8) with fuliginous tinge towards center, ochraceous (1B6-8) outwards, the olivaceous color stays at least around the center finally. LAMELLAE 2.5–4.5 mm broad in mid-radius, adnexed, crowded, when young cream white (1A1-2) when young, then grayish white (1B3) and discoloring to brown (4B1) with whitish edge in age; edge not smooth. STIPE 28–50 × 1.5–3.0 mm, equal with a swollen base, solid, longitudinally pruinose over the entire length except for the base, pale cream color (2A2-3), whitish at the apex and base. CONTEXT fleshy, in pileus 2.5–3.5 mm thick under the umbo, white (1A1), greenish near the pileipellis, odor spermatic; in stipe shiny, white (1A2), pinkish (5A2-3) near the surface, striate.

BASIDIOSPORES (8.7–)9.0–10.0(–10.6) × (5.8–)6.0–7.5(–7.8) µm, conspicuously nodulose, brown, Q = (1.20)1.24–1.55(1.58). BASIDIA 22–31 × 7–10 µm, clavate, with 4 sterigmata, clamp connections present at the base. PLEUROCYSTIDIA abundant, 46–82 × 13–21 µm, thick-walled (2–3.5 µm thick), yellow, crystalliferous at apex, mostly fusiform to narrowly fusiform, at times with a short to long neck, tapered in lower part, with clamp connections at base. CHEILOCYSTIDIA 41–70 × 10–18 µm, abundant, similar to pleurocystidia; PARACYSTIDIA abundant, often catenate, thin-walled, almost hyaline, terminal cells clavate to obovoid, rarely subglobose, 9–23 × 5–12 µm. CAULOCYSTIDIA 37–62 × 8–21 µm, descending to the base of stipe, similar to pleurocystidia but

FIG. 4. *Inocybe olivaceonigra* in situ in a Yunnan *Castanopsis* forest.

with thinner walls; CAULOPARACYSTIDIA 12–22 × 6–13 µm, obovoid or ellipsoid. HYMENIAL TRAMA composed of subregularly arranged, hyaline, inflated hyphae 12–18 µm wide. PILEIPELLIS a cutis, duplex, the upper layer indistinct pinkish in color, up to 130 µm thick, composed of subregular, cylindric hyphae 3–5 µm in diam., almost hyaline; the subtending layer golden yellow, up to 68 µm in thickness, composed of smooth to incrusted, inflated hyphae 8–20 µm in diam. CLAMP CONNECTIONS present in all tissues, but not at every septum.

HABITAT AND ECOLOGY: scattered and common on the ground in forests dominated by *Castanopsis*. July to September in Yunnan, China. Type recorded from *Castanopsis* forests at high elevations in Papua New Guinea.

SPECIMENS EXAMINED: **CHINA, YUNNAN PROV., KUNMING:** Qiongzhu temple, alt. 2100 m, under *Castanopsis*, 26 Jul 2011, T. Bau & Y.G. Fan 2011131 (HMJAU 24616; GenBank JX025775); 26 Jul 2011, X. Jin 2011131a (HMJAU 24617); 26 Jul 2011, Q.X. Guo 2011131b (HMJAU 24618); 26 Jul 2011, S.S. Yang 2011131c (HMJAU 24619); 26 Jul 2011 T. Bau 2011131d (HMJAU 24620; GenBank JX025776); 21 Sep 2006, Y.C. Li 717 (HKAS 51154). **PAPUA NEW GUINEA, MOROBE DISTRICT**, Bulolo, Heads Hump, alt. 1200 m, under *Castanopsis*, 9 Mar 1972, E. Horak (ZT Myc11128 [= ZT 72/192], holotype).

COMMENTS: The authors have examined the holotype of *Astrosporina olivaceonigra* (ZT Myc11128). The dried material is in good condition with two and a half basidiomata. The pilei are olivaceous-green around the center, paler outwards; the lamellae are apricot to yellowish brown, and the stipes are buff.

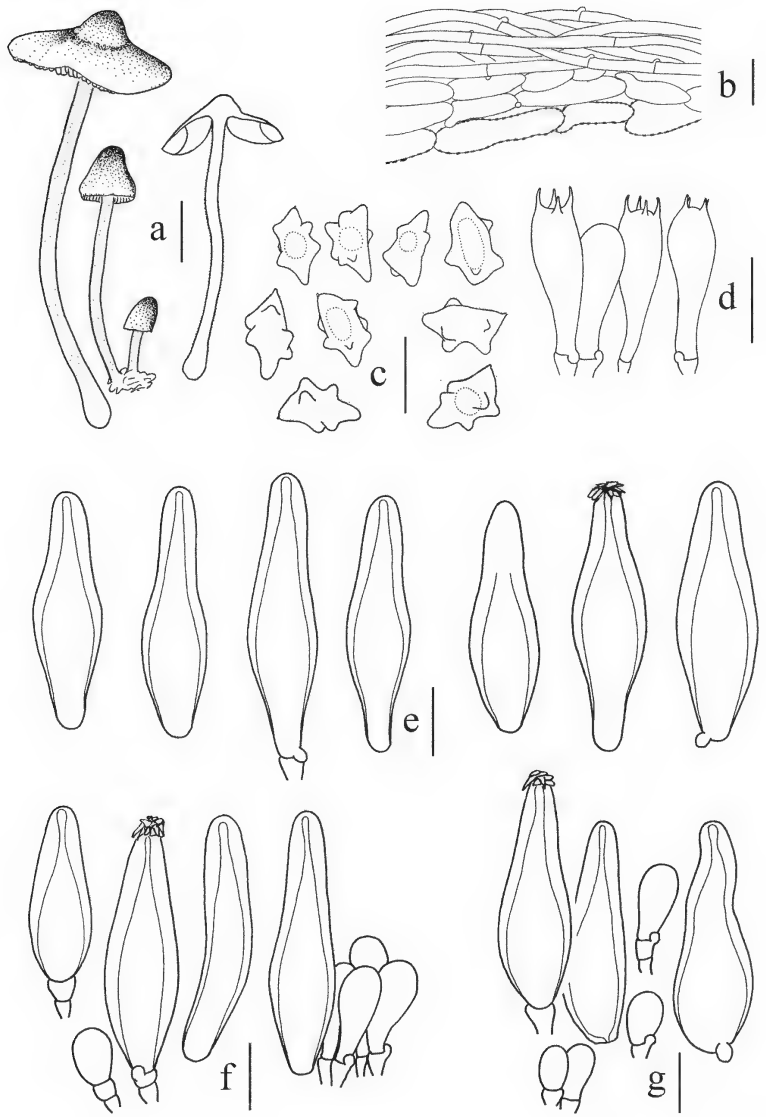

FIG. 5. *Inocybe olivaceonigra* (HMJAU 24620). a. Basidiomata; b. Pileipellis; c. Basidiospores; d. Basidia and sterile cell; e. Pleurocystidia; f. Cheilocystidia and paracystidia; g. Caulocystidia and cauloparacystidia. Scale bars: a = 10 mm; b–c = 10 μm; d–g = 20 μm.

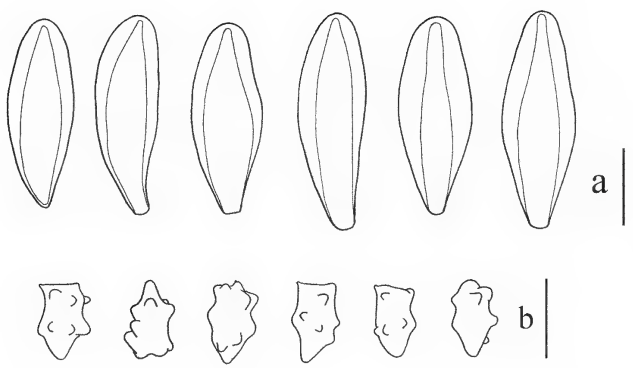

Fig. 6. *Inocybe olivaceonigra* (ZT Myc11128, holotype). a. Hymenial cystidia; b. Basidiospores. Scale bars: a = 20 μm; b = 10 μm.

Microscopically, the basidiospores (Fig. 6a) are nodulose with 9–11 rounded nodules ((7.7–)8.1–9.7(–10.7) × (5.3–)5.8–7.0(–7.8) μm, Q = (1.21–)1.34–1.55(–1.58)); hymenial cystidia (Fig. 6b) are fusoid (33–54 × 11–15 μm) with walls up to 4.5 μm; caulocystidia are similar to those of hymenial cystidia, but have thinner walls. Although Horak (1979) emphasized the conspicuous brown intra-hyphal pigments (in KOH) in cuticle hyphae in his type description, we observed that the hyphae in upper pileipellis layer in the holotype are identical to those of Chinese materials, and the subtending layer is pale yellow and composed of inflated and incrusted hyphae. The Chinese basidiospores exhibit a slightly more prominent knob at the apex, and most Chinese basidiomata possess hymenial cystidia with a thinner wall and a slender appearance (as in Fig. 5), but HMJAU 24616 and two basidiomata of HMJAU 24617 possess hymenial cystidia that agree well with the holotype (as in Fig. 6a). In view of the highly similar gross morphology, habitat, and ecology, we prefer to ignore any divergence at the species level. The two new ITS sequences (HMJAU 24616: GenBank JX025775; HMJAU 24620: JX025776) differ by a single base.

Inocybe glaucodisca Buyck & Eyssart., originally described from Zambia and which greatly resembles *I. olivaceonigra* in the glaucous grayish umbo, an entirely pruinose stipe, nodulose basidiospores, and thick-walled cystidia, differs by a finely squamulose pileus disc, a stipe base with a marginate bulb, and different habitat and ecology (Buyck & Eyssartier 1999). A recently described species, *I. pyriformis* Takah. Kobay. & S. Kurogi, which also has a smooth pileus, entirely pruinose stipe with a non-marginate base, and nodulose basidiospores differs in its dull red- to brown-colored stipe and pyriform to ventricose pleurocystidia (Kobayashi 2009). *Inocybe insignis* A.H. Sm., originally described from North

America is similar in having an entirely pruinose stipe, stellate basidiospores, and thick-walled cystidia; it shares the greenish color only after bruising or cutting. However, *I. insignis* has a larger habit, an aromatic odor, and larger stellate basidiospores (Smith 1941). *Inocybe viridiumbonata* Pegler differs by its robust habit, thin-walled cheilocystidia, slightly thick-walled pleurocystidia, and larger basidiospores. In addition, *I. viridiumbonata* occurs in a degraded xerophytic forest in the Caribbean (Pegler 1983).

BLASTn results of our *I. olivaceonigra* ITS sequences show 10 matches (94% similarity) labeled *I. suaveolens* D.E. Stuntz based on materials from North America, among which the holotype sequence of *I. suaveolens* (HQ222010) produced by Matheny is also included and three matches (93%-94% similarity) labeled *I. umbratica* Quél. based on materials from both North America and Europe. Morphologically, *I. suaveolens* (Smith & Stuntz 1950) and *I. umbratica* (Kobayashi 2002) can be easily distinguished from *I. olivaceonigra* by their lack of olivaceous colors on the pileus. The three species, however, do share the silky-smooth pileus, the entirely pruinose stipe, nodulose basidiospores, and thick-walled hymenial cystidia.

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Stipitate hydnums of the southern United States 1: *Phellodon mississippiensis* sp. nov.

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Abstract—During a general survey of fleshy fungi from Mississippi, an undescribed *Phellodon* species was observed in the Tombigbee National Forest at the same location over a two-year period. No other studies of the stipitate hydnums are available for the midsouthern states that directly compare with the new species. Molecular sequence data of *P. mississippiensis* were distinct from other *Phellodon* spp. of the southeastern United States. The highly fused (concrescent) basidiomata of *P. mississippiensis* are found under mixed hardwoods and associated pines. The light tan to light rust (orange) or salmon color on the marginal spines and along the margin of the small (≤5 cm broad) stout spongy tomentose basidiomata makes this species distinct. The presence of rare clamp connections was observed in the trama hyphae. However, identification of *P. mississippiensis* can be complicated by its concrescent nature and the indeterminate growth forms caused by environmental conditions. Morphological characters and results from the phylogenetic analysis are included in the species description.

Key words—white spored, mycorrhizal, early summer, fenugreek odor

Introduction

The stipitate hydnums in the southeastern United States have been studied extensively either directly or in larger monographs (Banker 1906, 1913a,b,c; Coker 1919, 1926, 1927, 1939, 1942; Jones 1935; Coker & Beers 1951; Baird 1986a,b, 1987; Baird & Kahn 1986, 1987). However, no specific studies of the group have been conducted for the mid-south, including Mississippi. David Lewis (Gulf States Mycological Society, pers. comm.) reports that many species of fleshy fungi have been identified and new species recorded in numerous published research papers from the state. Lewis stated that F.S. Earle & L.M. Underwood spent time along the Gulf Coast in the Ocean Springs, MS, area, collecting primarily *Amanita* and *Boletus* spp. Many of these species were

sent to W.A. Murrill, who published many as new. More recently W.G. Cibula extensively documented fleshy fungi for Mississippi (Cibula 1979, Cibula & Weber 1996), sending most collections sent to the Field Museum, Chicago, IL. Finally, of the three stipitate hydnums cited in Dukes' (2000) book on Mississippi fleshy fungi, two were correctly identified to genus as *Hydnellum* and another listed as *Phellodon* was incorrectly identified and was actually a wood decay fungus.

A study of the stipitate hydnums from southern Appalachian Mountains represents the most recent monograph on stipitate hydnums from the southern region (Baird 1986a). During that research, a thorough type study was also conducted with worldwide implications (Baird 1986b). More recently revision and phylogenetic analyses (unpublished data) of stipitate hydnums have shown that nine *Phellodon* spp. from Great Smoky Mountains and the surrounding southern Appalachian Mountains are distinct.

During a general survey of macrofungi in northern Mississippi, an unknown *Phellodon* sp. (Fig. 1) was collected from the same location during 2009 and 2010. The collections were found under mixed hardwood with conifers near the site. Morphological comparisons with all other known *Phellodon* spp. from southeastern United States indicated that none of the general herbarium collections or from previous type studies were similar (Baird 1986b). Furthermore, a BLAST search of molecular sequences from other *Phellodon* spp. from the southeastern United States and GenBank revealed no matches, supporting the species as undescribed. We present here a detailed description of the new species and its phylogeny (Fig. 3).

Materials & methods

In 2009 and 2010, collections were obtained from the North Trail sect. D of Noxubee Crest Mountain Bike Trail System of Tombigbee District of Tombigbee National Forest. Survey of associated tree species was conducted within a 30 m radius of the basidiomata.

All macro- and microscopic data were analyzed according to Baird & Kahn (1986). Colors were determined with Kornerup & Wanscher (1978). Digital images were taken of slide mounts of basidiospores and basidia using a Jenoptik ProgRes 3.3 megapixel digital camera mounted on a Olympus BX50 compound microscope. Basidiospore, basidium, and sterigma sizes were measured from these images using image-analysis software (IMT i-Solution Lite), and 20–40 measurements were made for each of three variables per collection. Scanning electron microscope (SEM) photographs were made of dried specimen material mounted on aluminum stubs with double-sided sticky carbon tape, coated with 15 nm platinum, and examined with a JEOL JSM 6500F SEM. The accelerating voltage was 5 KV and the images were digitally recorded.

Molecular examination

The nrITS DNA region from the new species was sequenced for phylogenetic comparisons with other *Phellodon* species. Genomic DNA was extracted from dried

basidiomata tissue using DNeasy, Plant Mini Kit by Qiagen (Valencia, CA) following procedures discussed in the brochure (08/2000) instructions. The ITS primers ITS1F (Gardes & Bruns 1993) and ITS 4 (White et al. 1990) were used to amplify the internal transcribed spacer region ITS 1-5.8S-ITS 2. Amplification reaction for ITS used procedures previously described (Lickey et al. 2007). The PCR products were purified with Qiagen PCR columns (Qiagen, Chatsworth, CA) and sequences by Eurofins-MWG/Operon (Huntsville, AL). Resulting electropherograms were viewed and edited using DNAstar of Lasergene (Madison, WI), and multiple sequences per taxon were collapsed due to lack of variation. When electropherograms were unreadable due to multiple sequences or insertion-deletions that could not be resolved, PCR products were cloned using pGEM-T vector system and SM 109 competent cells following manufacturers protocol (Promega). Sequences generated include *Phellodon* sp. 1 (TENN65891 = JN135199), *P. alboniger* (Peck) Banker (TENN65868 = JN135206), *P. brunneoolivaceus* (Coker & Beers) R.E. Baird (TENN65882, JN135201), *P. confluens* (Pers.) Pouzar (TENN65874= JN135198), *P. ellisianus* Banker (TENN65884= JN135204), *P. fuligineoalbus* (Fr.) Baird (in press) (TENN65893= JN135196), *P. fibulatus* K.A. Harrison (TENN65865= JN135205), *P. melaleucus* (Sw. ex Fr.) P. Karst. (TENN65896= JN135197), *P. niger* (Fr.) P. Karst. (TENN65876= JN135202), *P. putidus* (G.F. Atk.) Banker (TENN65883= JN135200), and *P. tomentosus* (Fr.) Banker (TENN65889= JN135203). Sequences of *Sarcodon atroviridis* (Morgan) Banker (TENN65871= JN135190), *S. imbricatus* (L.) P. Karst. (TENN65890= JN135194), and *S. scabripes* (Peck) Banker (TENN65894= JN135191) were designated as outgroup. All sequences were subjected to GenBank program BLAST to detect similar sequences, resulting in the inclusion of six additional sequences in the data set: *Phellodon* sp. 352-363 (AB509722), *P. nothofagi* McNabb PDD89898 (GU222318), *P. nothofagi* PDD89880 (GU222316), *P. atratus* K.A. Harrison (HQ650766, FN185793), and *P. sinclairii* (Berk.) G. Cunn. (GU222291). Sequences of the new species were submitted to GenBank and accession numbers JN247563 (MS-1) and JN247564 (MS-3) were designated.

The sequences were aligned using the web version of MAFFT (Katoh et al. 2005) with the G-INS-i option, which is provided by the CBRC (http://mafft.cbrc.jp/alignment/server/). The resulting alignment of 1,105 characters was used directly in jModeltest (Posada 2008) to determine TrN+G as the best fitting model of molecular evolution under the AICc. A Bayesian phylogenetic analysis was performed on the data set using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The three *Sarcodon* sequences were designated as outgroup. Using a GTR+G model the MrBayes analysis was conducted for 1 million generations, after which the split standard deviation reached 0.006. A burn-in of 2,500 trees was used prior to determining the posterior probability of the trees with the highest likelihood. The consensus tree is reported with posterior probability indicated as support for clades.

Taxonomy

Phellodon mississippiensis R.E. Baird, L.E. Wallace & G. Baker **sp. nov.** FIGS 1–2

INDEX FUNGORUM IF550077

Differs from *Phellodon putidus* and *Phellodon tomentosus* by its salmon colored spines, very stout fused pilei or stipes, and presence of clamp connections.

Type: United States of America. Mississippi, Noxubee County, Tombigbee National Forest, North Trail sect. D of Noxubee Crest Mountain Bike Trail System, (35°44.350′N 83°25.415′E), June 15, 2010, P. Scott, MS-3 (**holotype**, TENN65879; GenBank, JN247564).

Etymology: The epithet refers to the State in which the type was collected.

Basidiomata single, usually highly concrescent, ≤5.0 cm broad, stout, convex to plane or subdepressed or rarely colliculose; margin blunt in immature specimens to thin or irregular in age; spongy-tomentose becoming matted or appressed at disc, smooth to pitted or irregular at disc in aged tomentum, rarely radially rugulose, white (5A1) to orange white (5A2) or light orange (5A4) nearest margin, light brown (6D5), cinnamon (6D6) to burnt umber (6F6) or dark brown (6F7) at disc; underside of pileus margin and immature spines white (5A1) to pale orange (5A3) or light orange (5A5); context ≤3.0–4.0 mm thick nearest stipe, evidence of zones, concolorous with pileus. Stipes ≤1.5 × 2.0 cm concrescent or <1.0 × 1.0 cm single, central to eccentric, flattened or irregular on a side, tomentose becoming matted in age, bruised surface irregular, cylindrical to subattenuate above, at lower third entire base often fused forming a mycelial mass, lower portion of mycelial mass buried forming a thick network in the duff layer, white (5A1) nearest base to pale orange (5A3) or dark brown (6F6); context ≤1.5 cm thick, not obviously duplex, azonate, concolorous with pileus context. Spines up to 2.0 mm long, subcrowded, subdecurrent, white (5A1) to light orange (5A5) nearest margin. *Taste* none; *odor* moderate to strong fenugreek increasing upon drying. Chemical reaction in KOH light to dark brown on pileus context tissues over short period of time.

Pileus trama hyphae ≤5.0 µm diam, uninflated, generally interwoven in subsurface layer, parallel below, unclamped; rare occurring gloeophorous-like hyphae ≤7.0 µm diam, cylindrical to highly irregular-shaped. Stipe hyphae ≤4.5 µm diam, uninflated, interwoven in subsurface layer to parallel below, unclamped, rarely observed gloeophorous-like hyphae ≤9.0 µm diam, when present. Spine trama hyphae subparallel to parallel, ≤4.0 µm diam, rare clamp connections in subhymenial hyphae. Basidiospores 16.0–22.0 × 5.0–6.0 µm (mean = 17.9 ± 1.7 × 5.8 ± 0.5 µm), subglobose to globose, hyaline; ornamentation echinulate, spinules not prominent; hilar appendage oblique. Basidia 16.0–22.0 × 5.0–6.0 µm (mean = 17.9 ± 1.7 × 5.8 ± 0.5 µm), clavate, unclamped, 4-spored; sterigmata ≤4.0 µm long (mean = 3.6 ± 0.39 µm). Clamp connections absent in pileus and stipe contact tissues, rare in subhymenial hyphae of the spines.

Additional specimen examined: **UNITED STATES of AMERICA**. Mississippi, Noxubee County., Tombigbee National Forest, North Trail sect. D of Noxubee Crest Mountain Bike Trail System, (35°44.350′N 83°25.415′E), May 28, 2010, P. Scott, MS-1 (TENN65879; GenBank JN247563).

FIG. 1. *Phellodon mississippiensis*. a: Basidiomata showing upper portion of basidiomata; b: Basidiomata side view of fused pilei and stipes with thickened mycelial base. Scale bars = 5.0 cm.

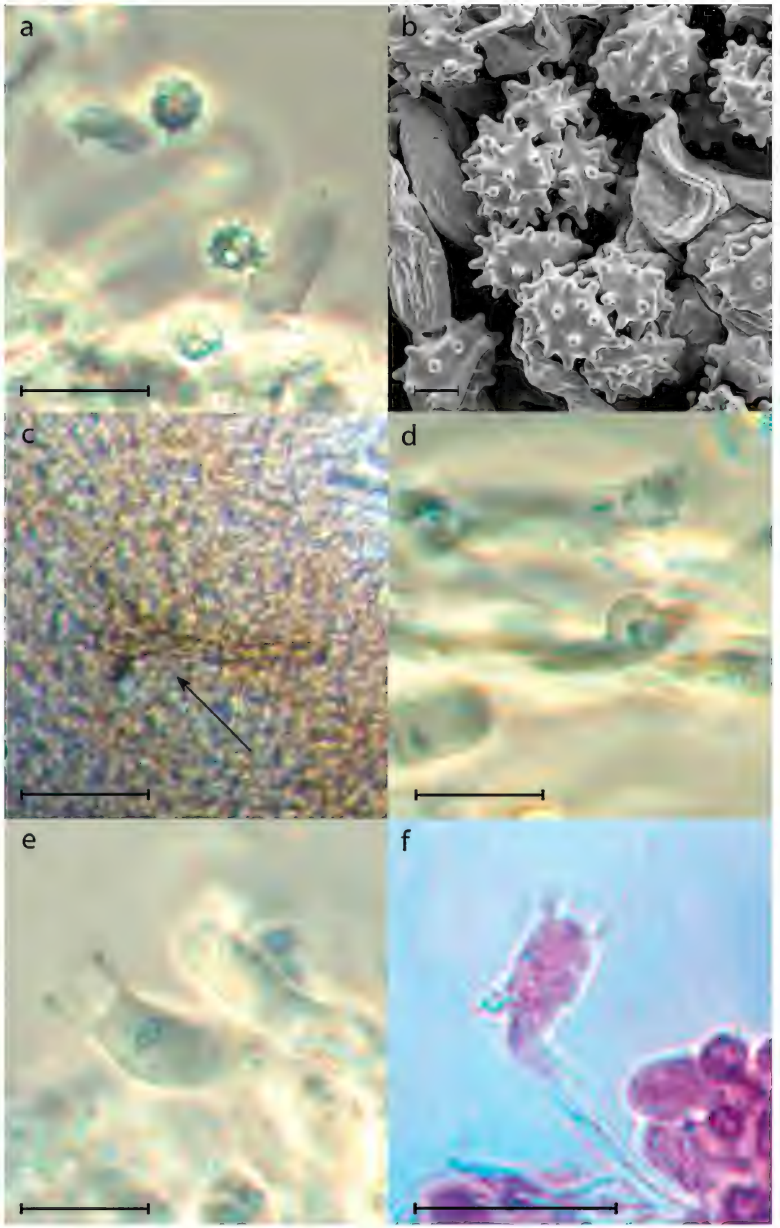

Fig. 2. *Phellodon mississippiensis*. a: Basidiospore with echinulate ornamentation; b: Scanning electron microscope (SEM) micrograph of basidiospores; c: Gloeophorous-like hyphae present in pileus and stipe; d: Clamp connection in the subhymenial hyphae; e–f: Basidia and sterigmata. Scale bar = 10 µm.

Habitat: The type location contained the following tree species (percent densities) within 17.4 m of the collections: *Pinus taeda* L. (20% = 5 trees), *Carya glabra* (Mill.) Sweet (16%), *Acer rubrum* L. (16%), *Fraxinus americana* L. (12%), *Cornus florida* L. (8%), *Oxydendrum arboretum* (L.) DC. (8%), *Ostrya virginiana* (Mill.) K. Koch (1%), *Quercus velutina* Lam. (1%), *Q. rubra* L. (1%), and *Nyssa sylvatica* Marshall (1%). The site (6.0% slope), which was 3.0 m from a small intermittent stream, also contained 30 year-old pines planted >25.0 m from the collections that were under the hardwoods.

Discussion

The phylogenetic analysis strongly supports *P. mississippiensis* as a member of the *Phellodon* clade, which is distinct from the outgroup *Sarcodon* species (100% PB; Fig. 3). The two *P. mississippiensis* samples are identical and unique within *Phellodon*, further supporting its separation as a new species. Within *Phellodon* however, the evolutionary relationship of *P. mississippiensis* with other species is unclear. Current phylogenetic analyses clearly place *P. mississippiensis* within *Phellodon* but are unable to resolve most sister species relationships within the genus (Fig. 3).

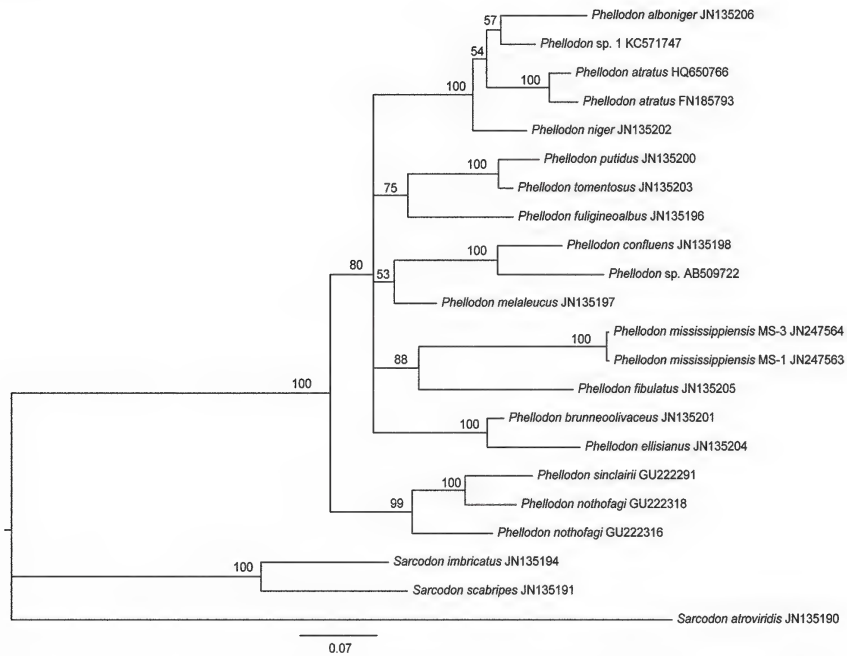

Fig. 3. Bayesian consensus phylogeny resulting from analysis of nrITS sequences. Values on branches are posterior probabilities and indicate support for nodes.

Molecular sequence data and morphological differences separate this species from all other *Phellodon* spp. known to occur in the southeastern United States, including Florida (Baird 1986a,b; Baird & Kahn 1986). Morphologically the basidiomata may be confused with several species based on indeterminate growth forms and concrescent nature, in particular, *P. fibulatus*, *P. confluens*, *P. tomentosus*, *P. radicatus* R.E. Baird and *P. putidus*. *Phellodon putidus* has marginal colors overlapping those of *P. mississippiensis* but is much larger (≤11 cm broad) and lacks the thickened fused stipe bases that can occur in *P. mississippiensis*. Basidiomata of *P. fibulatus* (with abundant clamp connections) and *P. tomentosus* (with clamp connections scattered randomly on stipe hyphae) are much thinner than *P. mississippiensis*. Unlike *P. mississippiensis*, *P. tomentosus* occurs only under conifers. *Phellodon confluens* has very thin basidiomata and lacks the light orange or salmon colors seen on the margin of *P. mississippiensis*. *Phellodon radicatus*, reported from Florida, has a blackish colored context and radicating stipe bases not present in *P. mississippiensis*, which is instead characterized by the thick mycelial mass formed from fused stipes and buried in the humus layer. Also, basidiospores of *P. radicatus* are 1.0 µm larger than in the new species.

Previous studies in Florida and surveys in the midsouth and along the Gulf Coast lead us to expect that new stipitate hydnum species will continue to be identified during surveys of this region.

Acknowledgements

Special thanks to the curators for supplying specimens from NYBG, MICH, NCSC, and TENN, and to Mississippi State University (MAFES publication number 12185) and Highlands Biological Station for use of their facilities and equipment during a long-term study of the *Phellodon* spp. from the southern United States.

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Two new species of *Lignosus* (*Polyporaceae*) from Malaysia — *L. tigris* and *L. cameronensis*

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Abstract —*Lignosus cameronensis* and *L. tigris* are described as new based on collections from the tropical forests of Pahang, Malaysia. Phenotypic and genotypic data support both as new species. Morphological features are illustrated, and the associated Internal Transcribed Region (ITS1 + 5.8S + ITS2) rDNA sequences have been deposited in the GenBank. Pore and basidiospore sizes are the main characters distinguishing these two *Lignosus* species from other members of the genus, with *L. tigris* distinguished from *L. sacer* by its larger pore size and smaller basidiospores and *L. cameronensis* separated from *L. ekombitii* by its smaller basidiospores. A key to the species of *Lignosus* is provided.

Key words — medicinal mushroom, sclerotia, taxonomy, phylogeny

Introduction

Lignosus Lloyd ex Torrend, a genus including six poroid species—*L. dimiticus* Ryvarden, *L. ekombitii* Douanla-Meli, *L. goetzii* (Henn.) Ryvarden, *L. hainanensis* B.K. Cui, *L. rhinocerotis* (Cooke) Ryvarden, *L. sacer* (Afzel. ex Fr.) Ryvarden—is known from the paleotropics (Cui et al. 2011, Dai 2012, Douanla-Meli & Langer 2003, Ryvarden & Johansen 1980). Members of this genus characteristically exhibit central stipitate basidiocarps, which rise from subterranean sclerotia. The genus is microscopically characterized by dimitic to trimitic hyphal systems, with dimitism currently reported only in *L. dimiticus,* which lacks binding hyphae in the context, sclerotium and stipe (Ryvarden & Johansen 1980). Apart from *L. goetzii,* basidiospores are known from all *Lignosus* species and are mostly hyaline, globose, isodiametric to ellipsoid depending on the species (Cui et al. 2010, Douanla-Meli & Langer 2003, Ryvarden & Johansen 1980).

Molecular analysis has supplemented conventional taxonomic classification of these macrofungi. Molecular markers used for *Lignosus* are the nuclear large subunit (nLSU), RNA polymerase II second largest subunit (RPB2), mitochondrial ATPase subunit six (ATP6) (Sotome et al. 2008), and the Internal Transcribed Region (ITS1 + 5.8S + ITS2) ribosomal DNA (Cui et al. 2010, Tan et al. 2010, Zhao et al. 2013, Tian et al. 2013). Easily amplified and possessing highly variable non-conserved ITS1 and ITS2 regions, the ITS region has proved useful for phylogenetic reconstruction of *Lignosus* species (Martin & Rygiewicz 2005, White et al. 1990).

Lignosus species have been widely studied due to their potential medicinal value. In Malaysia, locals regard the basidiocarps as important ingredients in traditional medicine, particularly for treating coughs and asthma, as well as for improving general well being (Chang & Lee 2004). Despite the promising benefits of these mushrooms, taxonomic research of *Lignosus* in Malaysia has been gradual and mediocre, with no significant records published since Ryvarden's discovery of *L. rhinocerotis* in the Penang island of Peninsular Malaysia (Ryvarden 1972).

Recent expeditions to the rainforests of Peninsular Malaysia have led to the discovery of two *Lignosus* species from the state of Pahang that conform to the characters of the genus but do not match any known species. We describe here these two new *Lignosus* based on morphological observations and molecular data and infer their phylogenetic relationships within *Lignosus*.

Materials & methods

Lignosus tigris specimens K and T were collected from a tropical forest in Lata Iskandar (4°17.46′N 101°34.41′E), Pahang, Malaysia on 12 June 2010; *L. cameronensis* specimens T1 and T8 were collected from the same forest on 17 July 2010. A small portion (50 mg) of sclerotia tissue was excised for maintaining a live culture and for DNA extraction; the remaining specimen was oven-dried for preservation as a herbarium specimen. *Lignosus tigris* K and *L. cameronensis* T1 herbarium specimens have been deposited at K(M) (Mycological Herbarium, Royal Botanic Gardens, Kew, UK); collections *L. tigris* T and *L. cameronensis* T8 are maintained in the personal herbarium of C.S. Tan, MARDI.

Morphological studies

Macroscopic characters are based on both fresh and subsequently dried material. Colors are based on the RGB color model, denoted by three numbers in brackets representing Red, Green, and Blue, respectively. Microscopic descriptions were carried out predominantly on dried specimens using a Nikon Z200 microscope with phase contrast illumination. Freehand sections were rehydrated in 70-95% ETOH prior to mounting in H_2O, 3% KOH, or Melzer's reagent. Basidiospores were observed and measured directly from the sections. Spore dimensions exclude the 5% extreme measurements from each end of the range given in parentheses. Q represents the mean length/width of the specified number of spores studied (n) (Largent 1977, Largent et al. 1977, Dai 2010).

Molecular taxonomy

DNA specimens were ground with fine sand powder, and DNA was extracted using a modified CTAB procedure (Cota-Sánchez et al. 2006). Polymerase Chain Reaction (PCR) amplification of the ITS was conducted in 25µl reaction volumes using the primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) suggested by White et al. (1990). The PCR parameters were as follows: initial denaturing at 94°C for 5 min, followed by 20 cycles of (94°C for 45 sec, 45°C for 45 sec, 72°C for 1 min), and final extension at 72°C for 5 min. Amplicons were analyzed through staining with ethidium bromide and 1% agarose gel electrophoresis, and subsequently purified using a glass-milk matrix (Fermentas, USA) according to the manufacturer's protocol. Purified PCR products were ligated into pGEM-T Easy vector (Promega, USA) prior to transformation of the *Escherichia coli* strain JM109. Four clones were sequenced in both directions using forward and reversed M13 sequencing primers via 1st Base Laboratories Malaysia (ABI system).

The resulting DNA electropherograms of the ITS region were truncated and assembled into consensus sequences via ChromasPro V1.41 (Technelysium Pty Ltd). Multiple sequence alignments were generated with the incorporation of several sequences from the GenBank, and subsequently encoded in a NEXUS format using ClustalX V2.0 (Larkin et al. 2007). Details of samples used in this study are summarized in Table 1

Table 1. *Lignosus* and *Daedaleopsis* samples used in molecular analyses.

Sample	Locality	GenBank No.	DNA source
L. cameronensis T1 (holotype)	Malaysia	JQ409483	Sclerotium
T8 (isotype)	Malaysia	JQ409484	Sclerotium
L. ekombitii	Cameroon	(ex Douanla-Meli)	Sclerotium
L. hainanensis	China	GU580884	Sclerotium
L. rhinocerotis Ryvarden 11324	Malaysia	(ex Ryvarden)	Pileus
CH2	Malaysia	FJ380871	Sclerotium
CH31	Malaysia	FJ899143	Sclerotium
L. sacer Ryvarden 11329	Zimbabwe	GU001674	Pileus
K(M) 1037	Kenya	GU001675	Pileus
L. tigris K (holotype)	Malaysia	JQ409481	Sclerotium
T (isotype)	Malaysia	JQ409482	Sclerotium
D. confragosa (Bolton) J. Schröt	Germany	FR686551	—
D. sinensis (Lloyd) Y.C. Dai	—	FJ627256	—

Maximum parsimony (MP) and maximum likelihood (ML) analyses were used in the phylogeny construction via PAUP 4.0b10 (Swofford 2003). The MP tree was inferred through a heuristic search procedure using 1000 bootstrapping replications, with 100 random sequence addition and tree bisection reconnection (TBR) branch swapping. All characters were unordered and weighted equally. The best-fit HKY + G model was selected using hLRT ($\alpha = 0.01$) through Modeltest v3.7 (Posada & Crandall 1998), and implemented for the ML analysis. Parameters were similar to the MP analysis, but with 100 ML bootstrap replicates instead.

Bayesian inference (BI) was conducted using Mr. Bayes 3.2.1 (Huelsenback et al. 2001, Ronquist & Huelsenbeck 2003). Best-fit models were generated by Kakusan v.3 using the Bayesian Information Criterion (BIC) (Schwarz 1978) (Tanabe 2007). BI analysis

was performed using the Markov Chain Monte Carlo (MCMC) method over a run of 2,000,000 generations, with a tree sampled every 100 generations. Chain stationarity was determined graphically using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/), and the first 20,000 trees were conservatively discarded as burn-ins prior to the construction of the final 50% majority rule consensus tree. The resulting phylogenetic inferences were analysed and processed via Figtree (Morariu et al. 2008).

Taxonomy

Lignosus tigris Chon S. Tan, **sp. nov.** PLATE 1

MYCOBANK MB 800843

Differs from *Lignosus sacer* by its larger pores and smaller basidiospores.

TYPE: Malaysia. Pahang, Lata Iskandar, 4°17.46′N 101°34.41′E, in tropical rain forests, 12 June 2010, Tan CS 'K' (**Holotype**, K(M) 177826; GenBank, JQ409481). Tan CS 'T' (**isotype**, MARDI; GenBank, JQ409482).

ETYMOLOGY: *tigris*, from the local folklore that the species arose from milk that dripped onto the ground while a tigress fed her cubs.

BASIDIOCARP annual, single, terrestrial, centrally stipitate with an isodiametric pileus; pilei up to 11.3 cm in diameter and 0.5 cm thick at the center; tough, without odor and taste. PILEUS obtusely umbonate, slightly depressed, concentrically zonate and cinnamon brown (169, 79, 5) to ochre (223, 161, 49), margin wrinkled, thin, slightly folded and becoming crisp when dry. Pore surface cream (249, 218, 161) to pale brown (234, 152, 4), pruinose when fresh. Pores roughly pentagonal or hexagonal, coherent. more or less honeycomb-like, 1–2 per mm, gradually smaller and denser towards the margin. Tubes layered, somewhat polygonal, apparent and deep, up to 4 mm depth; tube walls gradually thinning, becoming corky or crisp upon drying. Pileal context white (255, 245, 231), tough, up to 5 mm thick, turning cream (249, 218, 161) when dry. STIPE single, caramel brown (186, 81, 2), up to 14 cm long, 1–1.8 cm thick, slightly contorted; enlarged, rough and wrinkled at the base, gradually thinning and smoother towards the pileus. Context from stipe white (255, 249, 171), turning cream (249, 218, 161) when dry. SCLEROTIUM irregular, light-weight, wrinkled and soiled, up to 7 cm long and 6.5 cm broad. Context pure white (236, 236, 236) to cream (249, 218, 161), wrinkled, floccose-cottony to tomentulose around the core.

HYPHAL SYSTEM trimitic; all hyphae negative in Melzer's Reagent. Generative hyphae with abundant clamp, diameter less than 1.4 μm, hyaline and thin-walled, interwoven with flexuous, aseptate skeletal hyphae, 2.6–3.1 μm in diameter, within the hymenium. Binding hyphae common, branched, ranging from almost straight to tortuous, up to 2.3 μm in diameter. Trama composed predominantly of thick-walled skeletal hyphae, up to 3.4 μm diameter, with narrow lumen; and occasional binding hyphae, hyaline, branched, up to 1.9 μm.

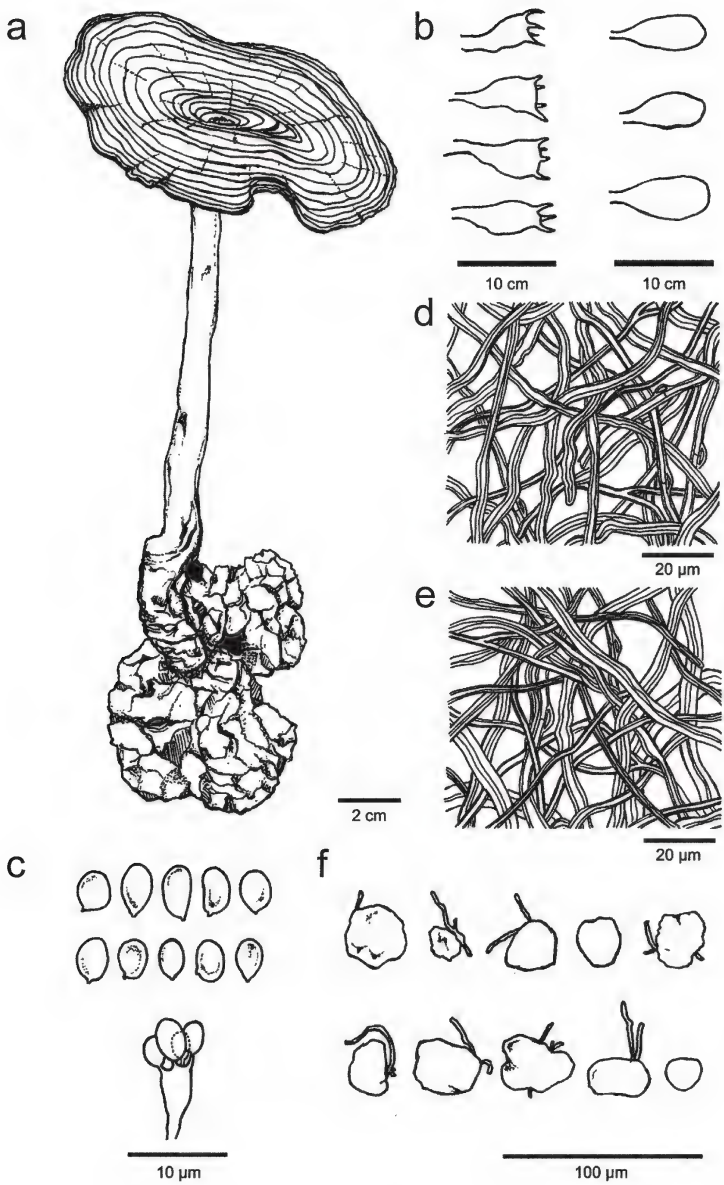

Plate 1. Microscopic structures of *Lignosus tigris* (holotype). **a** External morphology. **b** Basidia and basidioles. **c** Basidiospores and spore attachment. **d** Hyphae from trama. **e** Hyphae from stipe. **f** Sclerids.

Stipe context dominantly occupied by binding hyphae, hyaline, infrequently branched, diameter up to 1.9 µm; overlapped or slightly intertwined with sparse, slightly flexuous skeletal hyphae, hyaline, unbranched, diameter up to 4.7 µm, thick-walled with a narrow lumen. Sclerids dense and abundant within the sclerotium, subglobose, becoming somewhat oblong to irregular upon germination or when collectively clumped, 23.4–42.7 × 12.2–29.9 µm, margins wrinkled, appearing layered or stratified. Skeletal hyphae dominant, slightly sinuous, aseptate, hyaline, up to 3.5 µm in diameter, unbranched and thick-walled. Binding hyphae common, slightly flexuous, branched, interwoven, diameter up to 2.2 µm. Generative hyphae present, thin-walled, unbranched, hyaline, up to 1.5 µm in diameter.

Cystidia and cystidioles absent. Basidia clavate, subclavate, hyaline to granular, 4.9–9.1 × 1.75–3.3 µm; four sterigmata slender, generally 1.7–4.3 µm long, with some up to 4.3 µm long; basidioles mostly subclavate. Basidiospores mainly broadly ellipsoid to subglobose, infrequently globose and amygdaliform, (2.3–)2.5–5.5(–5.6) × (1.7–)1.8–3.6(–3.7) µm; Q = 1.34, (n = 44), thin-walled, hyaline, smooth.

Lignosus cameronensis Chon S. Tan, **sp. nov.** Plate 2

MycoBank MB 800844

Differs from *Lignosus ekombitii* by its smaller basidiospores.

Type: Malaysia. Pahang, Lata Iskandar, 4°17.46′N 101°34.41′E, in tropical rain forests, 17 July 2010, Tan CS 'T1' (**Holotype**, K(M) 177843, GenBank, JQ409483). Tan CS 'T8' (**isotype**, MARDI; GenBank, JQ409484).

Etymology: *cameronensis*, referring to Cameron Highlands, Malaysia; the locality of the type collection.

Basidiocarp annual, single, terrestrial, centrally stipitate with a more or less circular pileus; pilei up to 13.5 cm in diameter and 0.4 cm thick at the center; stout, without odor and taste. Pileus orbicular, slightly convex, brown (83, 32, 1) to chestnut brown (140, 55, 2), concentrically zonate, sulcate, rugose, margin deflexed, occasionally layered and overlapping; inrolled or incurved when dry. Pore surface cream brown (206, 157, 70), pruinose when fresh. Pores hardly visible, tiny and packed, isodiametric, 2–4 per mm. Tubes minute, dense, angular and layered; shallow, up to 2 mm deep; tube walls gradually thinning and becoming corky or crisp when dried. Tube layer up 4 mm thick. Context white (255, 245, 231), tough, up to 3 mm thick. Stipe muddy brown (175, 149, 97), single, central, equal, ellipsoidal, up to 8.5 cm long and 1.5 cm thick; woody hard and smooth when dry. Context from stipe cream (249, 218, 161). Sclerotium irregular, light, rugose and soiled, up to 6.5 cm long and wide. Context pure white (236, 236, 236), wrinkled and floccose-cottony around the center.

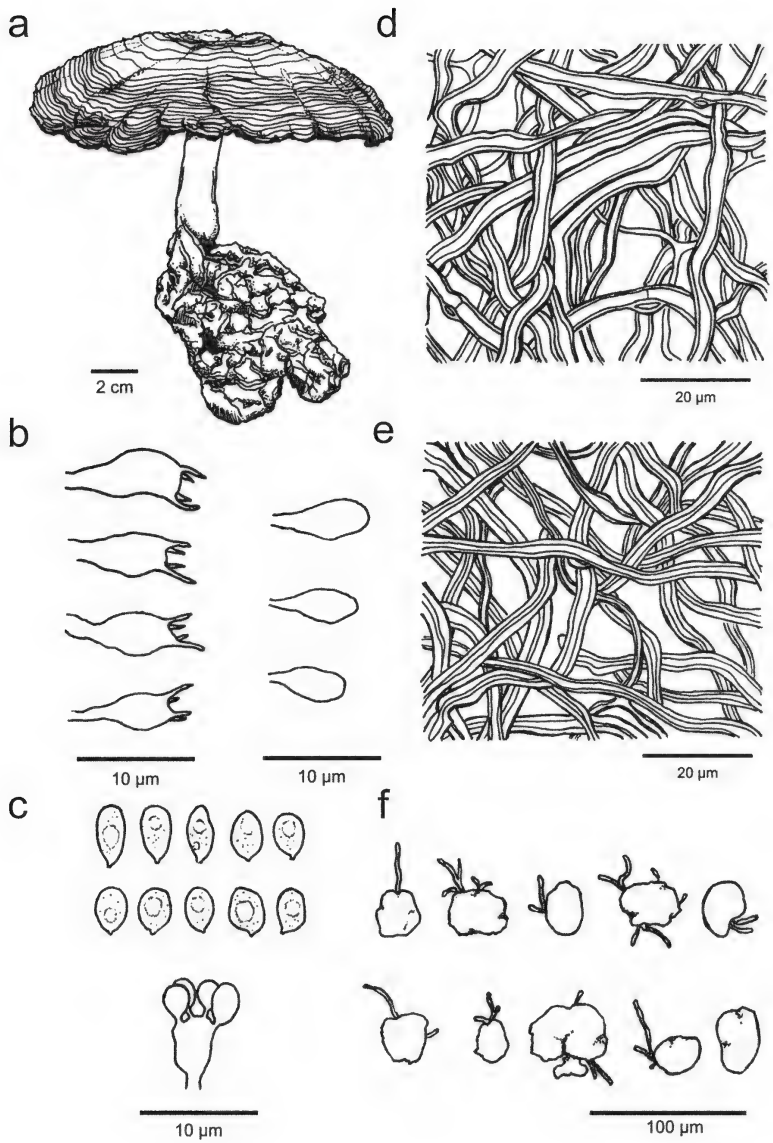

Plate 2. Microscopic structures of *Lignosus cameronensis* (holotype). **a** External morphology. **b** Basidia and basidioles. **c** Basidiospores and spore attachment. **d** Hyphae from trama. **e** Hyphae from stipe. **f** Sclerids.

Hyphal system trimitic; all hyphae negative in Melzer's Reagent. Generative hyphae with clamped connections, very irregular and contorted in the hymenium, thin-walled and hyaline, 1.2–1.5 µm in diameter; interwoven with occasional skeletal hyphae. Skeletal hyphae dominant within the trama, up to 3.6 µm in diameter, aseptate, thick-walled (up to 1 µm) with a narrow lumen, slightly flexuous, with scarce branching; mixed with thin-walled, almost straight to tortuous, aseptate binding hyphae, up to 2 µm in diameter; generative hyphae infrequent. Context of stipe, mainly of binding hyphae, integrating with relatively sparse, slightly flexuous skeletal hyphae; branching scarce. Sclerotium composed mainly of sclerids, originally subglobose or somewhat pear-shaped, becoming cordate to irregular upon germination or when clumped with other sclerids, 24.9–49.2 × 21.3–42.1 µm. Sclerid margins uneven, and seemingly stratified or striate. Skeletal hyphae dominant, thick-walled, hyaline, 1.3–4.1 µm in diameter, often branching. Generative hyphae less common, thin-walled, and unbranched, 1–1.6 µm in diameter. Binding hyphae present, interwoven, hyaline, often branching, 1.2–2.3 µm in diameter.

Cystidia and cystidioles absent. Basidia clavate, hyaline to granular, 3.6–8.9 × 1.4–4.5 µm; four sterigmata slender, generally 1.7–4.3 µm long, with some up to 5.7 µm long; basidioles mostly subclavate. Basidiospores mainly ellipsoid to subglobose, rarely globose, (2.2–)2.4–4.8(–4.9) × (1.8–)1.9–3.2(–3.4) µm; Q = 1.47, (n = 37), hyaline, smooth, thin-walled.

Molecular phylogeny

The ITS sequences were truncated at terminals to avoid ambiguous nucleotides, resulting in a length of 646 bp. Within the dataset, a total of 144 characters were considered parsimony-informative, whereas 466 characters remained constant. Parsimonious analyses of the ITS region resulted in two most parsimonious trees, with a consistency index (CI) of 0.902 and a retention index (RI) of 0.925. The nucleotide region encoding the 5.8S rRNA showed no variation among the *Lignosus* congeners. Conversely, large variations, including long indels were observed within the ITS1 and ITS2 regions. A genetic distance of up to 7.1% was observed between *L. tigris* and *L. cameronensis*. *Lignosus rhinocerotis* and *L. ekombitii* showed the highest genetic distance of up to 11.2% within the genus. An intraspecific genetic difference of 1.5% was observed between *L. cameronensis* T1 and T8. On the other hand, no intraspecific genetic variance was observed within the ITS region for the two *L. tigris* specimens.

The resulting phylogenetic tree (Plate 3) based on the ITS region indicates a clear distinction between the *Lignosus* ingroups and the *Daedaleopsis* outgroup. The suitability of this outgroup has been suggested and used in several studies (Cui et al. 2010; David & Michael 1995; Sotome et al. 2008). The monophyly (Clades A and B) of the *Lignosus* samples was inferred with high support

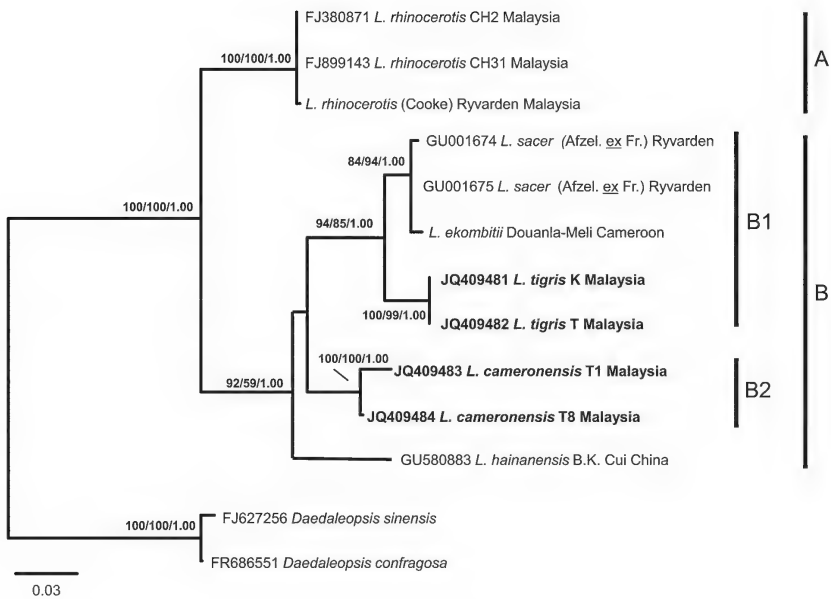

Plate 3. Maximum likelihood 50% majority-rule consensus tree based on the ITS region. Numeric values at nodes are arranged in the order of ML bootstrap support/MP bootstrap support/Bayesian posterior probabilities.

(ML = 100%; MP = 100%; BI = 1.00). The *L. rhinocerotis* sequences appeared as a sister group (Clade A) to the remaining *Lignosus* specimens. Clade B (ML = 92%; MP = 59%; BI = 1.00) is composed of *L. cameronensis* (Subclade B1); *L. tigris*, *L. sacer*, and *L. ekombitii* (Subclade B2); and *L. hainanensis*. The association between subclades B1 and B2 is currently unresolved. *Lignosus tigris* specimens (K and T) and *L. cameronensis* specimens (T1 and T8) each grouped with high support.

Discussion

Species currently placed in *Lignosus* generally have similar gross morphologies. Variations in size and dimensions of either sclerotia or basidiocarps are not characteristically unique to the species. Similarly, there is little variation between the hyphal systems, sclerids, etc. on a microscopic level. Currently, the sizes of the pores and basidiospores are the two characters sufficiently reliable to be used for species identification (Table 2). *Lignosus tigris* has strong similarities with *L. sacer*, and *L. cameronensis* with *L. ekombitii*.

Table 2. Pore and basidiospore sizes in *Lignosus*.

Species	Pores (per mm)	Basidiospores (µm)
L. goetzii	0.5–2	6–9 × 5–8
L. tigris	1–2	2.5–5.5 × 1.8–3.6
L. sacer	1–3	5–7 × 3–4.5
L. cameronensis	2–4	2.4–4.8 × 1.9–3.2
L. ekombitii	2–4	8.1–9.3 × 2.5–3.8
L. hainanensis	3–4	4.9–6 × 2.2–2.9
L. dimiticus	6–8	3–4.5 × 2.5–3
L. rhinocerotis	7–8	3–3.5 × 2.5–3

Lignosus tigris is differentiated from *L. sacer* by its larger pores (1–2 pores per mm) and smaller basidiospores (2.5–5.5 × 1.8–3.6 µm), and *L. cameronensis* differs from *L. ekombitii* by its smaller basidiospores (2.4–4.8 × 1.9–3.2 µm).

The phylogeny clearly separates *Lignosus rhinocerotis* from the other *Lignosus* species. All sampled *L. rhinocerotis* specimens clustered together with minor dissimilarities (<0.2% genetic distance), possibly resulting from spatial and time differences. The phylogenetic association between *L. cameronensis*, *L. sacer*, *L. ekombitii*, and *L. hainanensis* within Clade B remains unresolved with the ITS region molecular marker. This can eventually be addressed with longer gene regions, better molecular markers, and more specimens. The two *L. sacer* sequences (GenBank GU001674 and GU001675) differ slightly, with only two nucleotide substitutions at sites 69bp and 603 bp of the ITS region. The phylogenetic tree indicates that *L. ekombitii* is quite closely related, if not conspecific, with *L. sacer*; more specimens are needed to verify the identity of *L. ekombitii* phenotypically and genotypically. The high support for the monophylies of *L. tigris* and *L. cameronensis* confirms the morphological data that separate these from the other *Lignosus* species.

The molecular approach has thus far proved capable of inferring reliable phylogenetic relationships within *Lignosus*. More efforts in sequencing representatives of the remaining species and a broader sampling would help further elucidate this interesting genus.

Key to *Lignosus* species

1. Pileus white to ochraceous 2
1. Pileus light brown to dark brown 3

2. Pores large, 0.5–2 per mm ..... *L. goetzii*
2 Pores small, 6–8 per mm ..... *L. dimiticus*

3 Pores ≥7 per mm ..... *L. rhinocerotis*
3 Pores <6 per mm 4

4. Basidiospores broadly ellipsoid to subglobose 5
4. Basidiospores oblong ellipsoid to cylindrical 7

5. Basidiospores 5–7 µm long ..... *L. sacer*
5. Basidiospores 2.5–5.5 µm long 6

6. Pores 1–2 per mm ..... *L. tigris*
6. Pores 2–4 per mm ..... *L. cameronensis*

7. Basidiospores >6 µm long ..... *L. ekombitii*
7. Basidiospores <6 µm long ..... *L. hainanensis*

Acknowledgments

The authors would like to express gratitude to Dr. Leif Ryvarden for providing the specimens of *Lignosus rhinocerotis* and *L. sacer* and Dr. Clovis Douanla-Meli for the ITS DNA sequence of *L. ekombitii*. The authors thank Dr. Leif Ryvarden and Dr. Yu-Cheng Dai for the critical reviewing of the manuscript and constructive comments. Thanks are also due to Dr. Tan Swee Lian for the grammar checking and correction.

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Pionnotes, a synonym of *Dacrymyces* rather than *Fusarium*

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Abstract — The holotype of *Fusarium capitatum*, the type of the genus *Pionnotes*, was re-examined. Although *Pionnotes* was historically considered a synonym of *Fusarium*, it should henceforth be designated a synonym of *Dacrymyces*, with *F. capitatum* a synonym of *D. chrysospermus*. The generic name *Pionnotes* and species epithet *capitatum* should be evaluated further in future phylogenetic revisions of the *Dacrymycetales*, where most of the genera as currently understood are polyphyletic, and many common species such as *D. chrysospermus* may represent complexes of phylogenetic species.

Key words — *Hypocreales, Dacrymyces palmatus, Guepiniopsis,* taxonomy

Introduction

'Pionnotes' is an obscure term in the mycological lexicon. As a noun, it is mostly used in the taxonomy of *Fusarium* Link for colonies that have lost the discrete sporodochia that characterize the wild type. Its use as a descriptive term was probably proposed first in German by Appel & Wollenweber (1910: 28) as, "lagerartige Konidienansammlungen, die keine bestimmte Form haben und als Pionnotes mehr oder weniger ausgebreitete Schleime darstellen." By the time of the first international meeting of *Fusarium* taxonomists held at the University of Wisconsin in Madison in August 1924, pionnote was firmly established in the English terminology of *Fusarium* taxonomy (Wollenweber et al. 1925). In the modern literature, the term is still often used although, surprisingly, rarely explicitly defined (Booth 1971, Nelson et al. 1983, Gerlach & Nirenberg 1982, Burgess et al. 1994, Leslie & Summerell 2006). A pionnote is a continuous layer of slimy conidia, usually produced in culture but also sometimes in nature, essentially a broadly spreading sporodochium that lacks a fixed margin. On agar, such colonies usually lack aerial mycelium and are often considered mutants, or symptoms of cultural degeneration.

TABLE 1. Current status of other species attributed to *Pionnotes*, based on a review of the literature. *Fa* = *Fusicolla*. *Fm* = *Fusarium*.

Pionnotes sp.	CURRENT CLASSIFICATION	REFERENCE
P. betae (Desm.) Sacc.	≡ *Fa betae* (Desm.) Bonord.	Gräfenhan et al. 2011 [lectotype K(M) 167520!]
P. biasolettiana (Corda) Sacc.	?= *Fa merismoides* (Corda) Gräfenhan et al.	Wollenweber & Reinking 1935 (as *Fusarium*)[1]
P. capillacea Sacc.	?= *Dialonectria ullevolea* Seifert & Gräfenhan	Wollenweber & Reinking 1935
P. cesatii Sacc.	?= *Elsinoe ampelina* Shear	Wollenweber & Reinking 1935 [as *Gloeosporium ampelophagum* (Pass.) Sacc.]
P. ebulliens (Fr.) Sacc.	?= *Fm avenaceum* (Fr.) Sacc.	Wollenweber & Reinking 1935
P. flava (Fr.) Sacc.	type lost, identity unknown	Schroers et al. 2008
P. flavicans Sacc. & D. Sacc.	?= *Fm graminearum* Schwabe sensu lato	Wollenweber & Reinking 1935
P. navarrae Av.-Saccá	Unknown	no subsequent treatment
P. pinastri P. Karst.	?= *Linodochium hyalinum* (Lib.) Höhn.	Wollenweber & Reinking 1935
P. polysciadis Henn.	≡ *Cercosporella polysciadis* (Henn.) Hansf.	Braun 1995 (lectotype, B!)
P. pseudonectria Speg.	?= *Microcera coccophila* Desm.	Wollenweber & Reinking 1935
P. rhizophila (Corda) Sacc.	?= *Fa merismoides*	Wollenweber & Reinking 1935
P. sanguinea (Fr.) Sacc.	?= *Fm avenaceum*	Wollenweber & Reinking 1935
P. solani-tuberosi (Desm.) Sacc.	?= *Fm solani* (Mart.) Sacc.	Wollenweber & Reinking 1935
P. uda (Berk.) Sacc.	?= *Fa merismoides*	Wollenweber & Reinking 1935 (as *Fusarium*)
P. vagans Speg.	?= *Fm sambucinum* Fuckel	Wollenweber & Reinking 1935
P. violacea Lambotte & Fautrey	?= *Fm lateritium* Nees	Wollenweber & Reinking 1935
P. viridis Lechmere	= *Fm solani* sensu lato	Wollenweber 1916 (type illus., no. 418)

[1]The synonymies of Wollenweber & Reinking (1935) are considered tentative here, unless there is an indication in the series *Fusaria autographice delineata* (Wollenweber et al. 1916, etc.) that the type was examined.

The association of this term with *Fusarium* is not a coincidence; it is derived from the generic name *Pionnotes* Fr. (Fries 1849), generally listed as a synonym of *Fusarium* (e.g., Wollenweber & Reinking 1935). The generic name itself was derived from the Greek word *piōn*, meaning 'fat', referring to the texture of such colonies in nature. The 'Wollenweber system' of *Fusarium* taxonomy, while not accepting the genus, incorporated the name into section *Eupionnotes*

for several slow-growing species that typically produce such colonies. This section continued to be used in most taxonomic treatments of *Fusarium* until its species were transferred to *Fusicolla* Bonord. and other genera by Gräfenhan et al. (2011).

The putative but unconfirmed synonymy of *Pionnotes* with *Fusarium* was based partly on the origin of the basionym of the type species as *F. capitatum* Schwein. In his first treatment of hyphomycetes, Saccardo (1886) transferred nine additional species to *Pionnotes*. Although he did not alter the generic concept, he noted that, "Verisimiliter huc ducendæ sunt et aliæ Fusarii species." An additional eight species and one variety were added to *Pionnotes* by various authors after this influential compilation. Taxa attributed to *Pionnotes* are briefly reviewed in Table 1.

This paper presents observations on the holotype of *F. capitatum*, leading to the reconsideration of the generic name *Pionnotes* presented here. The surprising result shifts this name to the *Basidiomycota*, where it will need to be considered in phylogenetic reassessments of the generic concept of *Dacrymyces* Nees. This synonymy was reported cursorily by Gräfenhan et al. (2011) and Seifert et al. (2011); the details are published and discussed here.

This is the second of a planned series of papers on the typification and nomenclature of taxa that have been attributed to or confused with *Fusarium* (Seifert & Gräfenhan 2012).

Taxonomy

Pionnotes Fr., Summa Veg. Scand. 2: 481. 1849.

Type species: *Pionnotes capitata* (Schwein.) Fr., Summa Veg. Scand. 2: 481. 1849.

≡ *Fusarium capitatum* Schwein., Trans. Amer. Philos. Soc. 4(2): 302. 1832.

type: "3032 12 Syn. Fung., Fusarium capitatum Schwein. Penns." (Schweinitz Herbarium, PH 01089966, **holotype**).

The protologue of *F. capitatum* reads:

"3032. 12. F. capitatum, L. v. S., in ligno putrido Pini canadensis prope Factory, Pennsylv.

F. sporodochiis induratis, capitato-gyroso-expansis, (ut fere Tremella) ex aurantio rubris, majusculis, subcompresso-stipitatis. Aquae immersa, omnino solvuntur in sporidia majuscula, diaphana, teretia et vermiformia aut flexuosa, apicibus obtusatis."

In proposing *Pionnotes,* Fries (1849) paraphrased Schweinitz's diagnosis, but added no details, suggesting that he probably did not see a specimen himself:

"[LVI. Pionnotes. *Fr.*

Sporae majusculae, cylindricae, flexuosae, pellucidae in massam gelatinosam rigescentem (aurantio-rubram) effiguratam junctae. Typus Fusar. capitatum *Schw.* et pl. sp. exot.]"

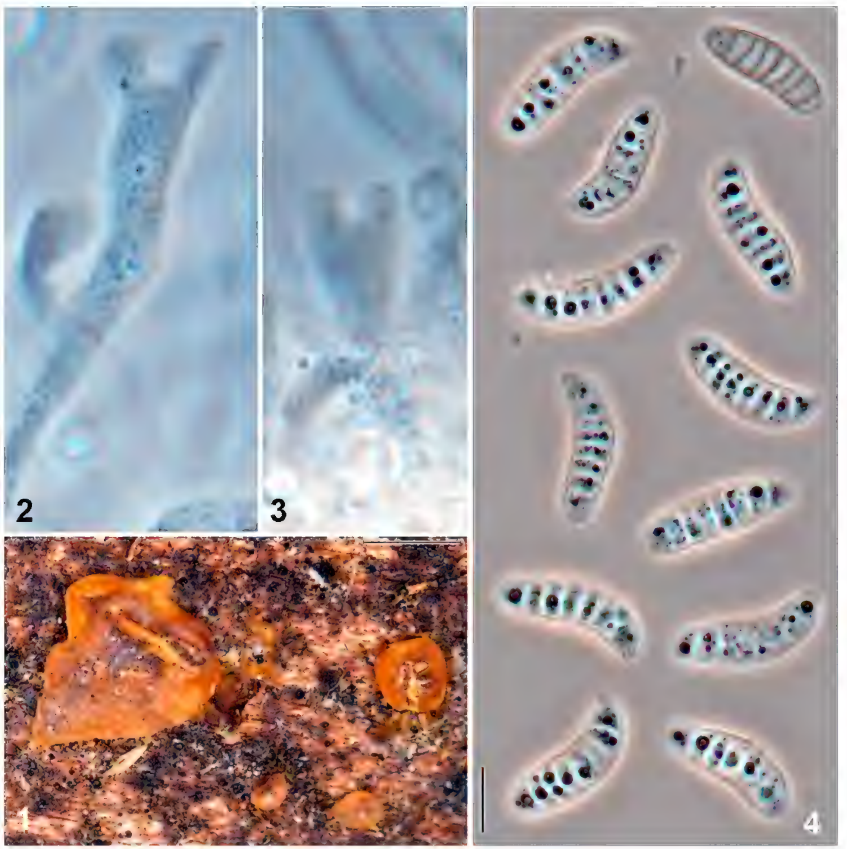
The type specimen of *F. capitatum* is a small piece of wood with several dry, hard, dark orange fruiting bodies of variable size (Fig. 1), the largest about 9 mm long and 4 mm wide. The smallest are more or less convex, but the larger are lobed and were probably originally cerebriform. When rewetted, the fruiting bodies are paler orange or yellow. Their surface is covered by what appears to be a dense hymenium with clavate probasidia that sometimes start to become bifurcate (Figs 2, 3). No mature basidia were seen. Abundant 7-septate spores (Fig. 4) cover the surfaces of the fruiting body. They are 20–25.5 × 6–7.5 µm, cylindrical to oblong and usually curved, with the most acute curvature near the base above a conspicuous papillate hilum.

The observations suggest that this is the common basidiomycete *Dacrymyces chrysospermus* Berk. & M.A. Curtis, as described by Kennedy (1956) and McNabb (1973), among others. This is a well-known species in eastern North America where the type of *F. capitatum* was collected, and it commonly grows on recently dead, fallen or cut *Tsuga canadensis*, the substrate reported by Schweinitz (as *Pinus canadensis*). When dry, the fruiting bodies of *D. chrysospermus* collapse from their hydrated, gelatinous state to hard, orange structures identical to those on the holotype of *F. capitatum*.

Discussion

Observations of the type specimen of *Fusarium capitatum* confirm that it is not a hyphomycete, but a basidiomycete identical to *Dacrymyces chrysospermus*. The taxonomic status of this epithet, and the genus it typifies, *Pionnotes*, must now be reevaluated.

In common with many classical fungal taxa, the pre-molecular generic concepts in the *Dacrymycetales* are challenged by new information. Shirouzu et al. (2007, 2009) used nuclear ribosomal large subunit (LSU) sequences to show that *Dacrymyces* as monographed by McNabb (1973) is polyphyletic. In their analyses, members of other morphologically defined genera, such as *Guepiniopsis* Pat., *Calocera* (Fr.) Fr., and *Dacryopinax* G.W. Martin, are intercalated in the *Dacrymyces* phylogeny. The clade including the type species of *Dacrymyces*, *D. stillatus* Nees, is separated from that including *D. chrysospermus* by *Guepiniopsis buccina* (Pers.) L.L. Kenn. The type species of *Guepiniopsis*, *G. tortus* Pat., is as yet unsequenced and the taxonomic consequences of this paraphyly are presently uncertain. Phylogenetic reevaluation of the generic concepts in the *Dacrymycetales* is forthcoming, and *Pionnotes* must now be considered as a potential name for the clade including *D. chrysospermus*, if it is to be recognized as distinct at the generic rank. Additionally, these published LSU phylogenies provide preliminary evidence for phylogenetic speciation within many morphologically defined species in this order, including *D. chrysospermus*.



Figs 1–4. *Fusarium capitatum*, holotype. 1. Basidiomata. 2, 3. Two probasidia becoming bifurcate. 4. Basidiospores (composite photograph). Scale bars: Fig. 1 = 1 mm. Fig. 4 (also 2, 3) = 10 µm.

Dacrymyces chrysospermus was known for many years, and is still often referred to in field guides, as *D. palmatus* Bres. The confusion over these two names requires some explanation. *Dacrymyces palmatus* was based on *Tremella palmata* Schwein. 1832, an illegitimate name (McNeill et al. 2012, ICN Art. 53.1) because of the earlier *Tremella palmata* Schumach. 1803. Bresadola (Höhnel 1904) transferred the epithet *palmata* to *Dacrymyces*, a nomenclatural act that legitimized it as a new name in *Dacrymyces* dating from 1904 rather than 1832 (ICN Art. 58.1). McNabb (1973) referred to both "*T. palmata* Schwein." and "*D. palmatus* (Schwein.) Bres." [sic] as "nom. nud.", his designation for what we now call illegitimate names. The next available name known to McNabb (1973) was *D. chrysospermus* Berk. & M.A. Curtis, the binomial adopted in

most subsequent academic literature. Considering the synonyms of *Dacrymyces chrysospermus* presented by McNabb (1973), *Fusarium capitatum* would be the earliest known legitimate name for this species. However, the epithet is already occupied in *Dacrymyces,* ironically by another name proposed in the same publication of Schweinitz (1832), *D. capitatus* Schwein. Thus, *F. capitatum* must be considered as a potential species epithet for phylogenetically delimited segregate species of the *D. chrysospermus* complex only if they are classified in a genus other than *Dacrymyces*.

In the absence of broader species sampling and multigene phylogenies for the *Dacrymycetales*, name changes would be premature and none are proposed here. Indeed, the long association of *Pionnotes* with the *Fusarium* literature may make it more prudent to reject this genus name as a potential source of error in interpreting historical literature, in the spirit of ICN Art. 57.1. This decision should be left to the taxonomic practitioners working with the *Dacrymycetales* or the mycologists now considering the creation of lists of protected or rejected names for inclusion in future Codes. This paper will serve its purpose by bringing these possibilities to their attention.

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A new species of *Stropharia* from Western Ghats, India

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Abstract—*Stropharia rubrobrunnea,* characterized by its caespitose habit, smooth grayish red or reddish to violet brown hygrophanous pileus, annular stipe, and dimorphic cheilocystidia, is described and illustrated as a new species. It is distinguished from *S. rugosoannulata* by its stipe color and basidiospore size.

Key words—*Agaricales*, *Basidiomycota*, dark-spored agarics, diversity, taxonomy

Introduction

The genus *Stropharia* (Fr.) Quél. (*Strophariaceae* Singer & A.H. Sm.) is poorly known from India, where only ten species have been recorded (Manjula 1983; Natarajan et al. 2005; Farook et al. 2013; Kumaresan & Senthilarasu, unpublished check list). Of these, *S. bicolor* Pegler, *S. rugosoannulata* Murrill (Manimohan et al. 2007), and *S. aurantiaca* (Cooke) M. Imai (Natarajan & Raman 1983) were recently recorded from southern India. Two excluded species are *S. pokhraensis* Dhanch. & Bakhukh. (Dhancholia & Bakhukhandi 1992), poorly known, and *S. semiglobata* (Batsch) Quél. (Natarajan & Raman 1983; Vrinda & Pradeep 2011; Mohanan 2011), which lacks true acanthocytes (Moncalvo et al. 2002) and has been transferred to *Protostropharia semiglobata* (Batsch) Redhead et al. The other poorly known species earlier recorded from India are *Stropharia aureofulva* (Berk.) Sacc. (as *Agaricus aureofulvus*), *S. gollanii* Henn., *S. mephistopheles* (Cooke) Sacc. (no material or illustration at Kew), *S. psathyroides* Henn., and *S. pygmaea* Henn. (Berkeley 1850, Hennings 1900). In the present paper, a new species collected from Western Ghats, *Stropharia rubrobrunnea,* is described, illustrated, and compared with closely related species.

Materials & methods

Thin handmade sections were made from dried specimens rehydrated with alcohol were mounted in 10% KOH and stained in 3% phloxine or cotton blue prior

to microscopical examination. Approximately 50 basidiospores were measured; the average spore range includes extreme values in parentheses. Colour terminologies follow Kornerup & Wanscher (1978). The type specimens are deposited at Ajrekar Mycological Herbarium (AMH), MACS' Agharkar Research Institute, Pune, India.

Taxonomy

Stropharia rubrobrunnea Senthil. & S.K. Singh, **sp. nov.** PLATE 1–3

MycoBank MB 564359

Differs from *S. rugosoannulata* by its white, smooth stipe and smaller basidiospores.

TYPE: India, Maharashtra state, Sinhgad, 18°21′56.39″N 73°45′18.97″E, 04.09.2011, coll. G. Senthilarasu (**holotype**, AMH 9447).

ETYMOLOGY: '*rubrobrunnea*' refers to the colour of the pileus.

PILEUS 6–18 mm (closed pileus) to 30–100 mm (matured pileus), hemispheric to broadly parabolic, not umbonate, slightly areolate, exposing white background when young; becoming convex to plane, slightly depressed, finally uplifted, broadly umbonate in the shallow depression; surface grayish red (8C4-9C4), dull red (8D4), reddish brown (9E7), changing to violet brown (10E7-10E8) to grayish red (11D5) when young, strongly hygrophanous, pinkish white (7A2-11A2-11A3) on orange white (5A2) ground, becoming yellowish white (4A2), moist, smooth; margin incurved, becoming decurved to plane, finally uplifted, irregularly appendiculate, fugacious, not striate, entire, becoming eroded. LAMELLAE adnexed to adnate, ≤5 mm wide, grayish red (11D4) to violet brown (11F4-11F5), becoming blackish brown, crowded with numerous lamellulae, eroded. STIPE central, 30–120 × 2–13 mm, terete, equal, slightly tapering towards base; surface white, shiny, smooth, longitudinally striate, cartilaginous to fibrous, solid, arising from white to yellowish white (4A2) rhizomorphs. ANNULUS membranous, white, becoming vinaceous, rugulose, attached at the one third of the stipe. PILEUS CONTEXT white, ≤3 mm thick.

BASIDIOSPORES (7–)7.5–8.5(–10) × (4.5–)5–5.5(–6.5) µm, (8.01 ± 0.36 × 5.47 ± 0.28) µm, Q = 1.46, ellipsoid, slightly rhomboid in face-view, brown with thickened dark wall, apically truncated by a broad germ-pore, smooth. BASIDIA 17–21 × 7–8 µm, cylindric clavate, tetrasporic; sterigmata ≤4 × 1.5 µm. LAMELLA EDGE sterile with crowded dimorphic cheilocystidia: CHEILOCHRYSOCYSTIDIA 19–38 × 6.5–11 µm, sublageniform with a short mucronate apex, with subhyaline to yellowish contents, thin-walled. CHEILOLEPTOCYSTIDIA 20–52 × 6.5–10 µm, clavate to cylindric with mucronate to rostrate apex; rostrum ≤30 × 2.5 µm, sometimes sphaeropedunculate, branched, thin-walled. CHRYSOCYSTIDIA abundant on the sides of the gills, 20–51 × 7–14 µm, lageniform with short mucronate apex, containing subhyaline to yellowish contents, thick-walled. HYMENOPHORAL TRAMA regular, with thin-walled, hyaline hyphae, 3–20 µm diam. SUBHYMENIUM well developed, ≤20 µm wide, pseudoparenchymatous.

Plate 1. *Stropharia rubrobrunnea* basidiomata under natural conditions: a. Young basidiomata. b. Mature basidiome showing rugulose annulus. c. Gill view of mature basidiome. (Photo Senthilarasu.)

Pileal surface a regular cutis of radially repent hyphae, 2–30 µm diam. Pileal context with hyaline hyphae 3–23 µm diam., thin-walled with clamp connections. Stipitipellis with hyaline hyphae 2–17 µm diam., thin-walled, clamped. Rhizomorph with sphaerocytes and hyaline hyphae, ≤7 µm diam, clamped; sphaerocytes ≤96 × 82 µm, globose to subglobose or ovate, hyaline, thin-walled; acanthocytes with several outgrowths, ≤83 × 3.7 µm, thick-walled, ≤1.7 µm thick, arising from a solid base, hyaline.

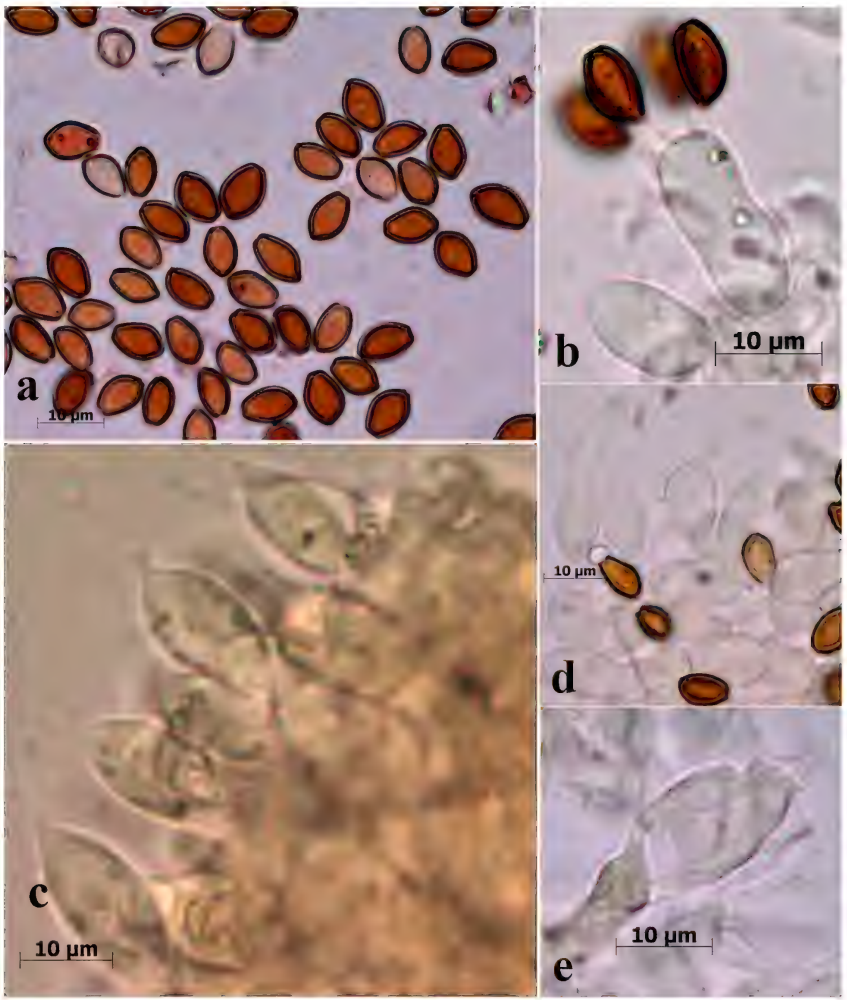

Plate 2. *Stropharia rubrobrunnea:* a. Basidiospores. b. Basidia. c. Cheilochrysocystidia. d. Cheiloleptocystidia. e. Pleurochrysocystidia.

Ecology & distribution: Solitary to caespitose to connate, on decayed gunny bag, in mixed forest vegetation. Known only from type locality.

The diagnostic features of *Stropharia rubrobrunnea* are the grayish red, reddish brown to violet brown pileus, white stipe with a membranous grooved annulus, and spores ≤10 µm long.

Recently, several *Stropharia* species have been described from the neotropical region (Cortez & Coelho 2004, 2008, Bandala et al. 2005, Silva et

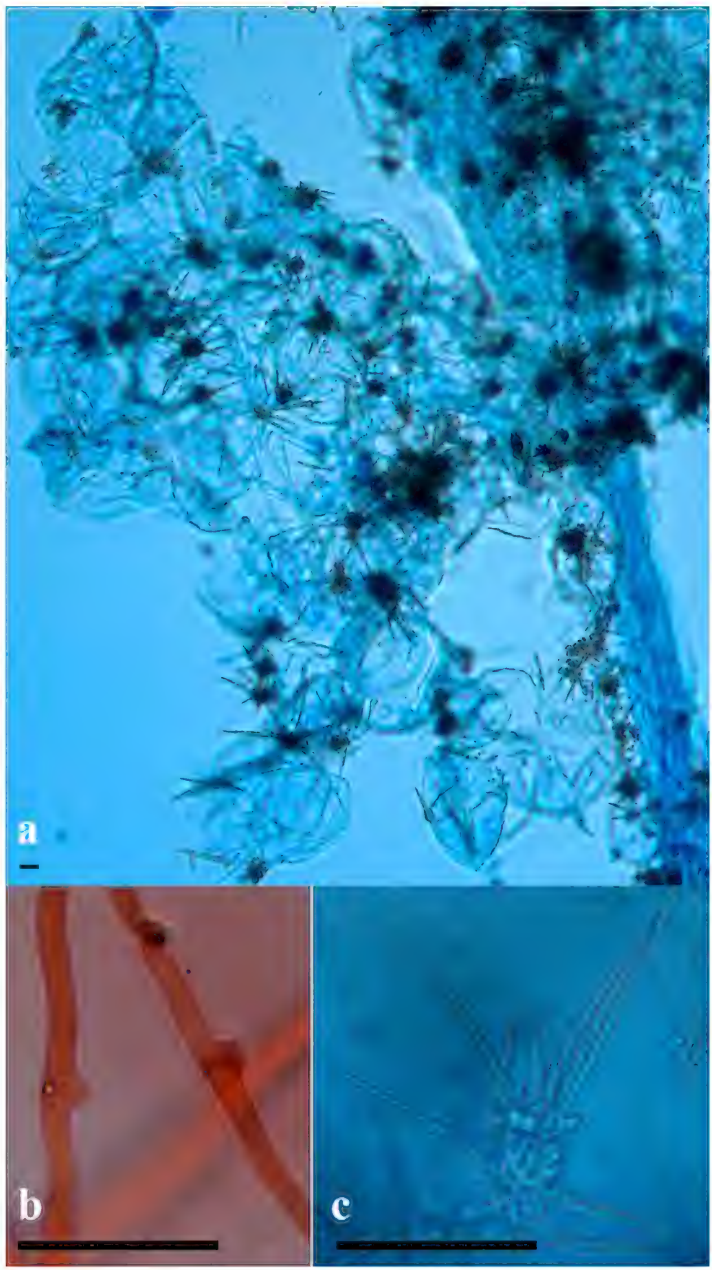

Plate 3. *Stropharia rubrobrunnea* rhizomorph: a. Sphaerocytes along with acanthocytes. b. Hyphae with clamp connections. c. Acanthocyte.

al. 2006, 2009, Cortez & Silveira 2007, 2008, Cortez 2008a,b) and China (Bau & Meng 2008). *Stropharia rubrobrunnea* resembles the most common species *S. rugosoannulata* (Cortez & Silveira 2008) in similar sized basidiomes. Although the pileus colour in *S. rugosoannulata* is highly variable, it has a yellowish brown stipe covered with brownish fibrils and larger basidiospores (9.5–14.5 × 6.5–8 µm) and basidia (21–38 × 8–12 µm).

Stropharia coronilla (DC.) Quél. and *S. araucariae* Cortez & R.M. Silveira (Cortez & Silveira 2008) also have a fleshy grooved annulus. Although *S. araucariae* also has leptocystidia and chrysocystidia at the gill edge, it differs from *S. rubrobrunnea* in its umbonate dark grayish brown pileus and larger basidiospores (10.5–13 µm long). The basidiospore size (6.5–10.5 × 4.5–6.5 µm) in *S. coronilla* is similar to that in *S. rubrobrunnea,* but it is a grassland inhabiting mushroom and differs morphologically in smaller basidiome size (pileus = 16–55 mm diam.; stipe = 18–51 mm long), yellowish colored pileus, and absence of cheilochrysocystidia.

The neotropical species *S. venusta* P.S. Silva et al., known only from *Araucaria angustifolia* forests of southern Brazil (Silva et al. 2009), also somewhat resembles *S. rubrobrunnea* in the larger basidiome size, grayish red to reddish brown pileus, and rugulose annulus. However, *S. venusta* clearly differs in its greenish grey colored stipe covered with scattered white squamules, larger basidiospores (11–15 × 6–10 µm) and chrysocystidia (35–70 × 7–16 µm), and absence of cheilochrysocystidia.

Stropharia variicolor Desjardin & Hemmes (Desjardin & Hemmes 2001) and *S. cifuentesii* Bandala et al. (Bandala et al. 2005) with their reddish to reddish brown colored pilei somewhat superficially resemble *S. rubrobrunnea*. *Stropharia variicolor* also has similar sized basidiospores (6.5–9 × 4.5–5.7 µm) but differs in smooth annulus, smaller cheiloleptocystidia (17.5–26 µm) that lack a rostrum, and an absence of cheilochrysocystidia. *Stropharia cifuentesii* clearly differs in its smaller basidiome size (pileus = 8–30 mm diam.; stipe = 9–37 mm long), fibrillose to squamulose pileus, whitish to yellowish brown lamellae, more slender stipe, absence of a fleshy annulus, smaller spores (5.5–7 µm), and absence of cheilocystidia.

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Two new species of *Endophragmiella* from Spain

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Abstract — Two new species of *Endophragmiella* are described and illustrated. *Endophragmiella bicolorata* is characterized by (1–)2(–3)-septate conidia with a dome-shaped hyaline to subhyaline apical cell and brown basal and middle cells, a pigmentation pattern not previously described for species of this genus. *Endophragmiella cantabrica* is distinguished from species with 1-septate conidia by oblong, ellipsoidal, or sometimes ovoid conidia comprising two equal and uniformly pale brown cells.

Key words — anamorphic fungi, plant debris, taxonomy

Introduction

Sutton (1973) established *Endophragmiella* for two species: *E. pallescens* B. Sutton, selected as type, and *E. canadensis* (Ellis & Everh.) B. Sutton, currently considered a synonym of *E. subolivacea* (Ellis & Everh.) S. Hughes. Hughes (1979) clarified the conidiogenesis in *Endophragmiella*, and amended the genus definition. Subsequently, Kirk (1985), Holubová-Jechová (1986), and Wu & Zhuang (2005) contributed to the knowledge of the genus with keys based on morphological revisions of the species.

Endophragmiella is characterized by simple or branched conidiophores, monoblastic percurrently proliferating conidiogenous cells, and conidia with very variable morphology and septation, seceding rhexolytically. The species are mostly saprobes occurring on rotten wood, dead branches, and decaying leaves of different plants. Currently, more than 80 species are accepted in the genus (Rifai 2008, Ma et al. 2011, Ren et al. 2011, Seifert et al. 2011).

During an extensive survey of anamorphic fungi on plant debris in the Iberian Peninsula, two *Endophragmiella* species were found. We propose these as new species based on conidial morphology, size, and pigmentation.

Materials & methods

Samples of plant material were processed using the methodology described by Castañeda-Ruiz (2005). Fungal specimens were studied and illustrated following the procedure described by Hernández-Restrepo et al. (2012). All attempts to isolate the specimens in pure culture failed, despite trying different media and culture conditions; therefore, only dried material is preserved. Specimens are deposited in the herbariums of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS) and Faculty of Medicine, Reus, Spain (FMR).

Taxonomy

Endophragmiella bicolorata Hern.-Rest., J. Mena, Guarro & Gené, **sp. nov.**

MycoBank. MB 800594 Figs 1, 2

Differs from *Endophragmiella oblonga* by the conidial cell pigmentation pattern and dome-shaped apical cell.

Type: Spain, Valencia, Km 21 Road CV 425, Los Pedrones direction to Buñol, 39°18′28″N 0°52′38″W, on dead wood, 15/3/2010, M. Hernández-Restrepo & K. Rodríguez (**Holotype**, CBS H-21042; **Isotype**, FMR 10965).

Etymology: Latin *bicolorata*, referring to the pigmentation of conidia.

Colonies on the natural substratum effuse, hairy, black. Mycelium mostly immersed, composed of septate, smooth, subhyaline to pale brown hyphae, 2–3 µm wide. Conidiophores macronematous, simple, erect, straight or slightly flexuous, smooth, septate, brown, paler towards the apex, up to 125 µm long, 2–5 µm wide at the base, with up to 9 percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapered to a truncate apex. Conidia acrogenous, solitary, simple, ellipsoidal to oblong ellipsoidal, sometimes obclavate or obovoid, (1-)2(-3)-septate, often constricted at the septa, basal and middle cells brown, apical cell hyaline to subhyaline and dome-shaped, smooth all over, 19–26.5 × 7.5–13 µm, with a distinct subhyaline basal frill, 1.5–4 × 2–3 µm. Teleomorph unknown.

Note: This species resembles *Endophragmiella oblonga* (Matsush.) S. Hughes in morphology and conidial size, but the *E. oblonga* conidia are less variable in shape (Matsushima 1975, Hughes 1979). Although both species have a dome-shaped apical cell, their pigmentation pattern distinguishes the two; in *E. oblonga* the middle cell is brown and the end cells are pale brown (Hughes 1979, Mel'nik 2000).

Other *Endophragmiella* species with 2-septate conidia somewhat similar to *E. bicolorata* are *E. collapsa* (B. Sutton) S. Hughes, *E. ontariensis* S. Hughes, *E. suboblonga* W.P. Wu, and *E. tripartita* S. Hughes. However, the conidia of *E. collapsa* are smaller (14.4–17(–20) × 7.2–8.3(–9) µm) and have pale brown to dark brown distal cells and a basal cell that is paler and sometimes collapsed (Sutton 1973, Hughes 1979, Wu & Zhuang 2005). In *E. ontariensis*, the conidia

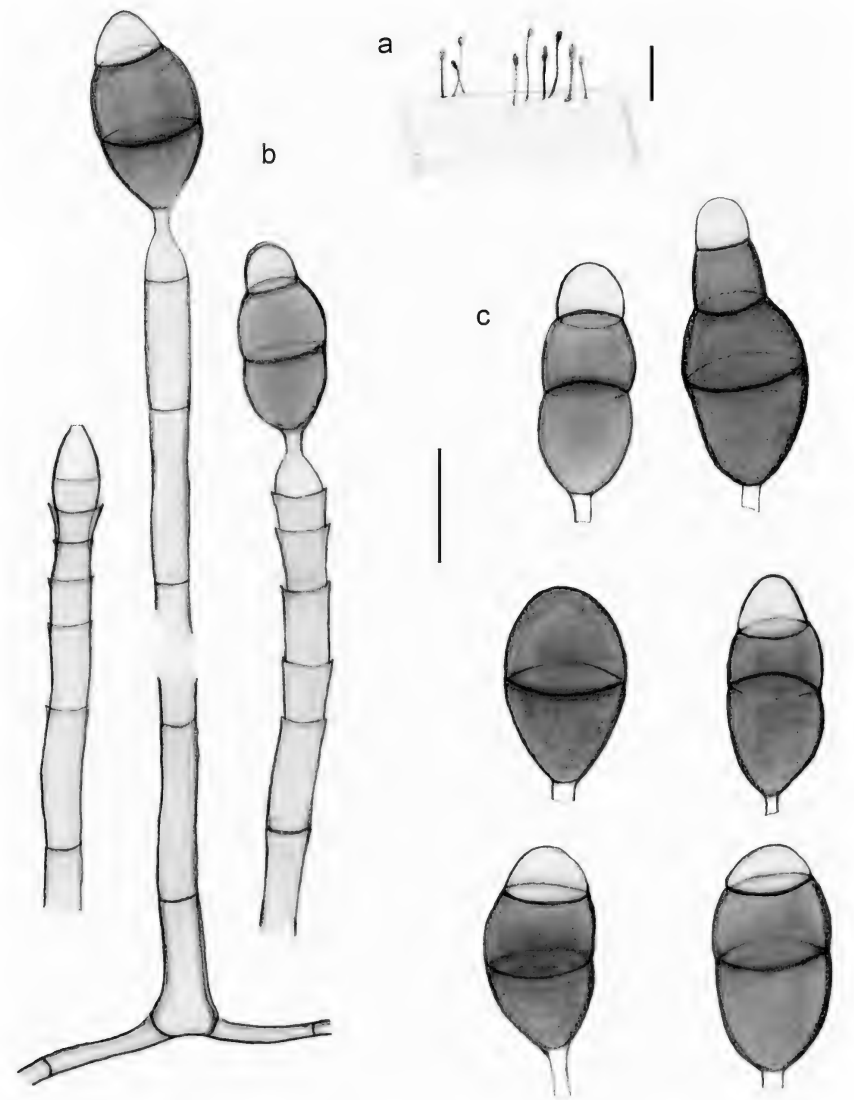

Fig. 1. *Endophragmiella bicolorata* (CBS H-21042). **a.** Habit. **b.** Conidiophores and conidiogenous cells producing conidia. **c.** Conidia. Scale bars: a = 100 µm; b,c = 10 µm.

are also smaller (18–22 × 8.5–11 µm) and have pale brown to dark brown distal cells and pale brown basal cells (Hughes 1978a, Wu & Zhuang 2005). The conidia of *E. suboblonga* measure 18–20 × 9–13 µm and have the middle

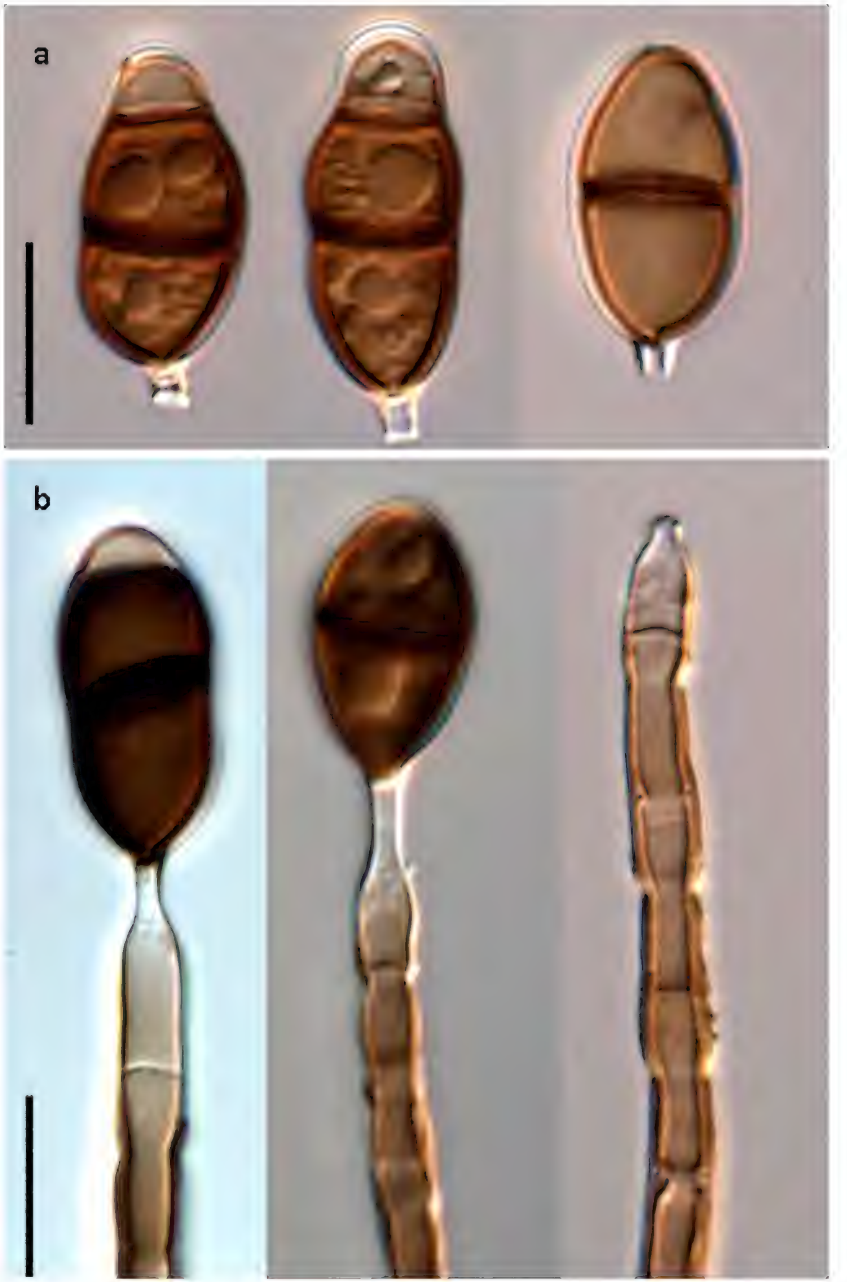

Fig. 2. *Endophragmiella bicolorata* (CBS H-21042). **a.** Conidia. **b.** Conidiophores, conidiogenous cells with conidia. Scale bars: 10 µm.

cell brown and larger and both apical and basal cells paler and smaller (Wu & Zhuang 2005). The conidia of *E. tripartita* measure (16–)18–21.5(–23.5) × 8.1–9.5 µm and have all cells brown to dark brown (Hughes 1979).

Endophragmiella cantabrica J. Mena, Hern.-Rest., Guarro & Gené, **sp. nov.**

MycoBank. MB 800595 Figs 3, 4

Differs from all other bicellular *Endophragmiella* species by its conidial size.

Type: Spain, Cantabria, Los Tojos, Barcená Mayor, Saja-Besaya Natural Park. 43°06′42.81″N 4°12′13.48″W, 675 m.a.s.l., on dead wood, 12/7/2010, M. Hernández-Restrepo, J. Mena & J. Guarro (**Holotype**, CBS H-21043; **Isotype**, FMR 11857).

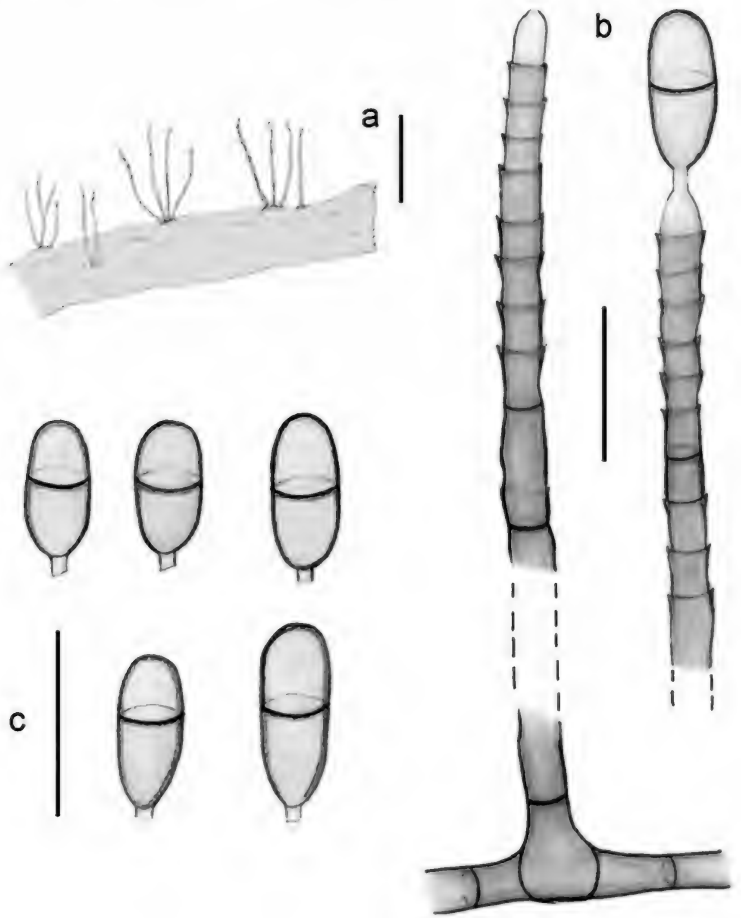

Fig. 3. *Endophragmiella cantabrica* (CBS H-21043). **a.** Habit. **b.** Conidiophores and conidiogenous cells producing conidia. **c.** Conidia. Scale bars: a = 200 µm; b,c =10 µm.

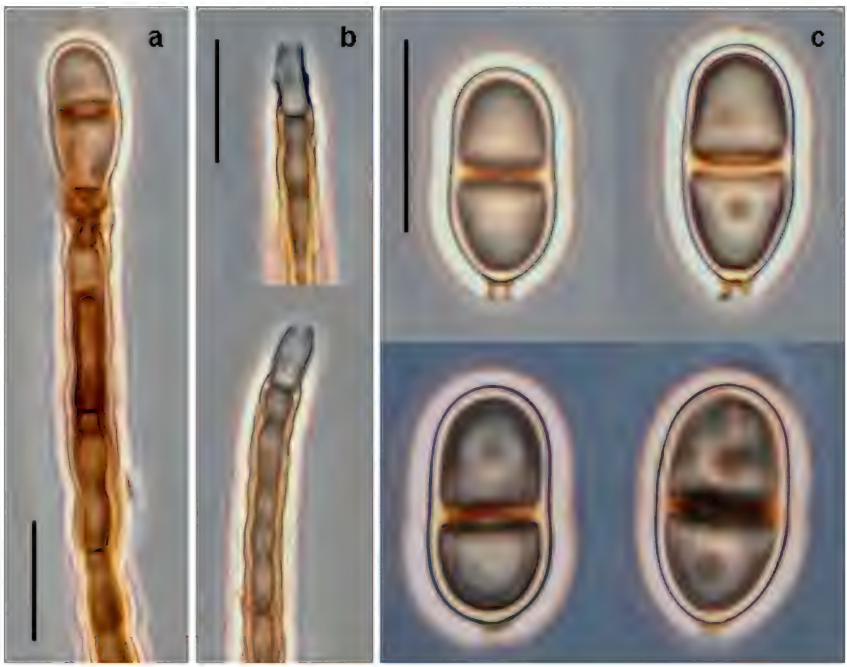

Fig. 4. *Endophragmiella cantabrica* (CBS H-21043). **a.** Conidiophore. **b.** Detail of conidiogenous cells. **c.** Conidia. Scale bars: 10 µm.

Etymology. Latin *cantabrica*, referring to the Spanish region where the fungus was collected.

Colonies on the natural substratum effuse, hairy, brown. Mycelium mostly immersed, composed of septate, smooth or verruculose, subhyaline to pale brown hyphae, 1.5–3 µm wide. Conidiophores macronematous, simple, erect, straight or slightly flexuous, smooth, septate, brown, paler towards the apex, up to 195 µm length, 5–6 µm wide at the base, with up to 20 percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapered to a truncate apex. Conidia acrogenous, solitary, simple, oblong, ellipsoidal, sometimes ovoid, 1-septate, pale brown, with both cells equal and uniformly pigmented, smooth, 10–14 × 5–6 µm, with a distinct subhyaline basal frill, 0.5–1.5 × 0.5–1.5 µm. Teleomorph unknown.

Note: Among the taxa of *Endophragmiella* with 1-septate conidia, *E. cantabrica* has some similarities with *E. arranensis* P.M. Kirk, *E. bogoriensis* Rifai, *E. ovoidea* P.M. Kirk and *E. uniseptata* var. *pusilla* Hol.-Jech. *Endophragmiella arranensis* has very short conidiophores (≤45 µm long after successive percurrent proliferations) and smaller conidia (6.5–9.5 × 4–5 µm) with the basal cell paler

than the apical one (Kirk & Spooner 1984). The other three species have more pigmented and larger conidia: 12–18 × 7–9 µm in *E. bogoriensis*, (10–)14–16 × (5–)5.5–6.5 µm in *E. ovoidea*, and (9–)15(–17) × (7–)9(–10) µm in *E. uniseptata* var. *pusilla* (Kirk 1981, Holubová-Jechová 1986, Mel'nik 2000, Rifai 2008).

Other *Endophragmiella* taxa with 1-septate conidia slightly similar to *E. cantabrica* in conidial morphology are: *E. angustispora* S. Hughes, *E. resinae* P.M. Kirk, and *E. uniseptata* (M.B. Ellis) S. Hughes var. *uniseptata*. Conidia of *E. angustispora*, however, are navicular to ellipsoidal or narrowly ovoid, 14.4–20.5 × 4.5–5.4 µm (Hughes 1978b); those of *E. resinae* are ovoid to pyriform, 17–22 × 9–10.5 µm (Kirk 1981); and those of *E. uniseptata* var. *uniseptata* are ellipsoidal, obovoid to pyriform, broadly obtuse at the distal end, 13–27 × 9–12 µm (Ellis 1959, Hughes 1979, Holubová-Jechová 1986).

Acknowledgments

The authors would like to thank Dr. Rafael F. Castañeda Ruiz, Dr. Cony Decock, and Dr. David Minter for their critical review of the manuscript. This study was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL 2011-27185.

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Sporisorium linderi, a new record for Asia

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Abstract — *Sporisorium linderi* (*Ustilaginomycetes*), reported here from Pakistan, is a new record for Asia. *Digitaria ciliaris* is a new host record for smut fungi in Pakistan.

Key words — KPK, *Poaceae*, taxonomy

Introduction

During exploration of smut fungi of Mansehra District, Khyber-Pakhtunkhwa (KPK) Province, Pakistan, *Digitaria ciliaris* was found infected with *Sporisorium linderi*.

The sampling site, located at 34°14′–35°11′N 72°49′–74°08′E, is bounded in the north by Batagram and Kohistan, in the east by the Muzafarabad district of Azad Jammu and Kashmir, in the south by Abbottabad and Haripur, and in the west by Swat and Kala Dhaka. The mountain ranges that enter Mansehra district from Kashmir are offshoots of the great Himalayan range. The district has a seasonal temperature range of 2–36 °C (Mustafa 2003).

Sporisorium Ehrenb. ex Link is the largest genus of smut fungi and is represented by 327 species (Vánky 2011, Denchev et al. 2013), of which 18 species have been recorded previously from Pakistan (Ahmad et al. 1997, Vánky 2007, Denchev et al. 2013).

Materials & methods

Freshly collected infected flowers/spikelets of *Digitaria ciliaris* were examined under a stereomicroscope. For light microscopic observation spores were mounted in lactophenol on glass slides and gently heated. Preparations were observed under a Nikon YS 100 microscope and photographed with the help of Digipro-Labomed. Spore

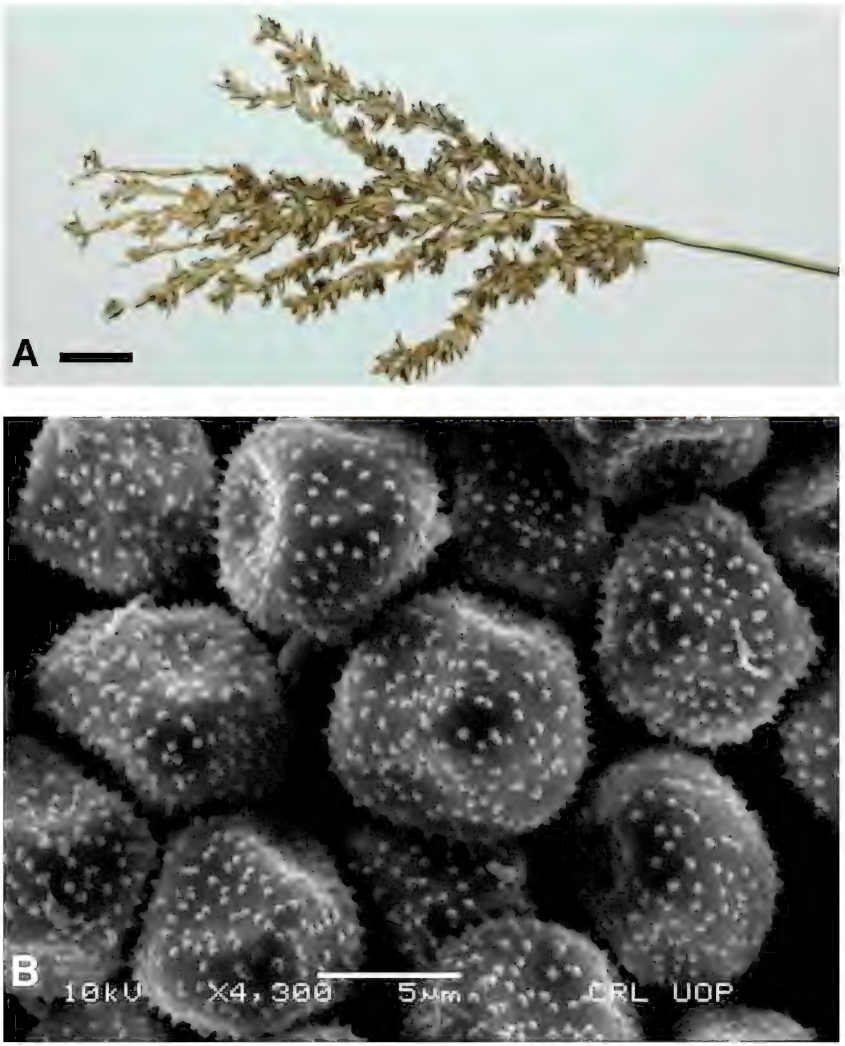

Fig. 1: *Sporisorium linderi* on *Digitaria ciliaris.* A. Infected inflorescence of the host plant. B. Spores in SEM. Scale bars: A = 1.2 cm; B = 5 µm.

measurements were taken by using an ocular Zeiss Eye Piece Screw Micrometer. For SEM, the spores were critically point dried, attached to specimen holders by double-sided adhesive tape, and coated with a 50 nm film of gold in a Polaron E5300 freeze drier. The gold-coated stubs were observed and photographed in a Camscan 3-30BM Scanning Electron Microscope.

Taxonomy

Sporisorium linderi (Zundel) Vánky, Mycotaxon 73: 142, 1999. FIG. 1

Sori in most of the spikelets of the inflorescence, mostly the basal spikelets are infected, sub-globoid to cylindrical, 1.7–3 mm long, initially covered by a thick peridium of the host origin which flakes away irregularly, exposing dark brown powdery spore mass and a well developed central columella, branched at tip, spore balls composed of loosely arranged cells. Spores globose to subglobose or ovoid to irregular, light olivaceous brown to somewhat darker, 7–9.2 × 8.2–10.4 µm, wall up to 0.7 µm thick, moderately to densely echinulate in LM, sparsely echinulate in SEM. Sterile cells either occur singly among the spores or in groups or chains, usually larger than the spores, hyaline, walls up to 0.4 µm thick, smooth.

SPECIMEN EXAMINED — On leaves of *Digitaria ciliaris* (Retz.) Koeler: **PAKISTAN**, **KHYBER-PAKHTUNKHWA**, Mansehra, Durbund, ca. 600 m, 3 Sep. 2010, M. Fiaz FS-24 (HUP 304).

COMMENTS: *Sporisorium linderi* is known from central and southern Africa on several *Digitaria* spp., including *D. ciliaris* (Vánky et al. 2011). This is the first record of its occurrence in Asia. Previously, ~50 % of the known *Sporisorium* species have been reported from Asia. This is the first report of a smut fungus on *Digitaria ciliaris* in Pakistan (Ahmad et al. 1997, Vánky 2011).

Acknowledgements

We sincerely thank Dr. Kálmán Vánky for his help in identification of the fungi and Dr. C.M. Denchev (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences) and Dr. Najam-ul-Sahar Afshan (Centre for Undergraduate Studies, University of the Punjab, Lahore) for their valuable suggestions to improve the manuscript and acting as presubmission reviewers.

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New combinations, *Scheffersomyces amazonensis* and *S. ergatensis*

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Abstract — On the basis of gene sequence analyses, two new combinations are proposed for the genus *Scheffersomyces* (*Saccharomycotina*, *Debaryomycetaceae*).

Key words — *Ascomycota*, xylose-fermenting yeasts.

Introduction

Based on recent radical changes to the rules governing the nomenclature of pleomorphic fungi (McNeill et al. 2012: Article 59), seven new combinations were made in the *Scheffersomyces* clade (*Saccharomycotina, Debaryomycetaceae*) supported by a multilocus phylogenetic analysis, and three new species were described in *Scheffersomyces* (Urbina & Blackwell 2012). Here we propose two additional new combinations in *Scheffersomyces* for species included in the same phylogenetic analysis, *Candida amazonensis* and *C. ergatensis*.

Taxonomy

Scheffersomyces amazonensis (Cadete, M.A. Melo, M.R. Lopes, Zilli, M.J.S. Vital, F.C.O. Gomes, Lachance & C.A. Rosa) Urbina & M. Blackw., **comb. nov.**

MycoBank MB 563718

≡ *Candida amazonensis* Cadete, M.A. Melo, M.R. Lopes, Zilli, M.J.S. Vital, F.C.O. Gomes, Lachance & C.A. Rosa, International Journal of Systematic and Evolutionary Microbiology 62: 1439, 2012.

Scheffersomyces ergatensis (Santa María) Urbina & M. Blackw., **comb. nov.**

MycoBank MB 801388

≡ *Candida ergatensis* Santa María, Anales del Instituto Nacional de Investigaciones Agrarias, Agricola, Madrid 1: 85, 1971.

Discussion

An ex-type culture of *Candida amazonensis* UFMG-HMD-26.3^T (= CBS 12363 = NRRL Y-48762) was included in the multilocus phylogenetic analysis (Urbina & Blackwell 2012), but a new combination for this taxon was not proposed because the species name had not yet been effectively published at the time of our revision. A second taxon included in our analysis and in the *Scheffersomyces* clade, *Candida ergatensis* NRRL Y-17652^T (= CBS 6248) was inadvertently omitted from our taxonomic revision (Urbina & Blackwell 2012).

Both *S. amazonensis* and *S. ergatensis* were isolated from rotted wood, and they share the ability to ferment glucose, galactose, and the disaccharide cellobiose, and assimilate other wood components such as D-xylose and D-arabinose (Santa María 1971, Cadete et al. 2012).

Acknowledgments

We thank Heide-Marie Daniel for calling our attention to the omission of *C. ergatensis* from the previous revision, as well as Scott Redhead and Cletus Kurtzman for nomenclatorial advice and manuscript review.

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McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ. 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum Vegetabile 154. 240 p. http://www.iapt-taxon.org/nomen/main.php

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Urbina H, Blackwell M. 2012. Multilocus phylogenetic study of the *Scheffersomyces* yeast clade and characterization of the N-terminal region of xylose reductase gene. PLoS ONE 7(6): e39128. http://dx.doi.org/10.1371/journal.pone.0039128

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Additional information on *Lecanora loekoesii*

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Abstract — Sixty-eight specimens of *Lecanora loekoesii* from China were collected for further study to supplement data derived from the South Korean type (previously the only known specimen). The ascospores of *L. loekoesii* often appear to be 1-septate because the protoplasm is divided. Four chemical types of *L. loekoesii* were discovered.

Key words — Asia, taxonomy, lichen, fungi

Introduction

The multispored species of *Lecanora* include eleven members worldwide: *L. bruneri* Imshaug & Brodo, *L. cateilea* (Ach.) A. Massal., *L. japonica* Müll. Arg., *L. loekoesii*, *L. pleospora* Müll. Arg., *L. polysphaeridia* Alstrup, *L. praesistens* Nyl., *L. sambuci* (Pers.) Nyl., *L. strobilinoides* Giralt & Gómez-Bolea, *L. subjaponica* L. Lü & H.Y. Wang, and *L. weii* L.F. Han & S.Y. Guo (Alstrup 1993, Guderley & Lumbsch 1999, Han et al. 2009, Lü et al. 2011, 2012). *Lecanora strobilinoides* is the only species where the ascospores are frequently 1-septate.

As a new species to science, *L. loekoesii* was described by only one specimen from South Korea (Lü et al. 2011). In the present paper, a more detailed and complete description of this species is given based on 68 specimens from China.

Materials & methods

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). The morphological and anatomical characters of the specimen were examined using a stereomicroscope (COIC XTL7045B2) and polarizing microscope (Olympus CX41-32). Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with C system (Orange et al. 2010). Photos of the lichen were taken under Olympus SZX16 and BX61 with DP72.

Results & discussion

Lecanora loekoesii Y. Joshi, L. Lü, & J.S. Hur, *Lichenologist* 43(4): 321–329 (2011)

FIGS 1–2

DESCRIPTION — The *L. loekoesii* specimens from China are characterized by the following characters: corticolous habit; thin continuous crustose thallus that is whitish gray to grayish green; soredia, prothallus, and pycnidium all lacking; indistinct cortex with crystals; lecanorine apothecium; yellowish brown apothecial discs with/without white pruina; amphithecium with indistinct cortex and small K-soluble crystals; epihymenium with coarse K-soluble granules; hyaline hymenium without oil droplets; hyaline hypothecium; simple non-pigmented paraphyses; 12–16-spored asci; simple or 1-septate-like (due to divided protoplasm) ellipsoid hyaline ascospores; and thallus spot tests of K+ yellow, KC+ yellow, C-, and P+ yellow.

Both the Chinese specimens and Korean holotype of *L. loekoesii* have the same epihymenium crystals. Lü et al. (2011) described the crystals in epihymenium of holotype as fine granules. However, we think these are better interpreted as coarse granules. Half of the *L. loekoesii* specimens from China have 1-septate-like ascospores because the protoplasm is divided, while the ascospores of the holotype are simple. We observed the 1-septate-like ascospores often in the same apothecium or even in the same ascus together with the simple ascospores.

We retested the lichen substances of the holotype by TLC; in addition to atranorin, usnic acid, zeorin and norstictic acid, we found three unknown lichen substances B, D, and E were found (FIG. 2). Of the 68 Chinese specimens tested, 51 have the same chemicals as the holotype, 14 specimens lack norstictic acid, two specimens have placodiolic acid, while one specimen has stictic acid plus an additional unknown substance J.

DISTRIBUTION IN CHINA — Heilongjiang, Jilin, Liaoning, Hubei, Shaanxi, Tibet.

SPECIMENS EXAMINED (except for the holotype, all conserved in SDNU): **Chemical type 1: SOUTH KOREA. GANGWON-DO**, Taebaek-si, Mungoksodo-dong, Mt. Taebaek, 37°06′48·8″N 128°56′51·0″E, alt. 910 m, on *Quercus* bark, 13 Oct 2005, Lőkös 050717 (holotype, KoLRI). **CHINA. HEILONGJIANG**, Wuchang, Mt. Datudingzi, alt. 1800 m, 21 Aug 2011, Y.L. Cheng 20122274B; alt. 1300 m, 21 Aug 2011, Y.L. Cheng 20122316B; alt. 1200 m, 21 Aug 2011, Y.L. Cheng 20122060A1, 20122233B, 20122249, 20122249D1, 20122273B, 20122288C; alt. 1100 m, 21 Aug 2011, Y.L. Cheng 20122088B, 20121912, 20122047, 20122149B, 20122220D, 20122394A; alt. 900 m, 21 Aug 2011, Y.L. Cheng 20127190; alt. 800 m, 21 Aug 2011, Y.L. Cheng 20122097, 20122155B, 20122155B1, 20122212B, 20122311A; Daxinganling, Mt. Daqing, alt. 800 m, 26 Aug 2011, Y.L. Cheng 20125519A; Heihe, 727 linchang, alt. 488 m, 16 Aug 2009, Q. Ren 20100966; Tahe, Mt. Baikalu, alt. 700 m, 24 Aug 2011, Y.L. Cheng 20125087; Tieli, Taoshan national forest park, alt. 900 m, 23 Aug 2011, Y.L. Cheng 20125116B, 20125738A, 20125154; alt. 800 m, 23 Aug 2011, Y.L. Cheng 20125355A1, 20125607B, 20125687E, 20125694B, 20125759B; alt. 700 m, 23 Aug 2011, Y.L. Cheng 20125310, 20125348A, 20125628A, 20125648,

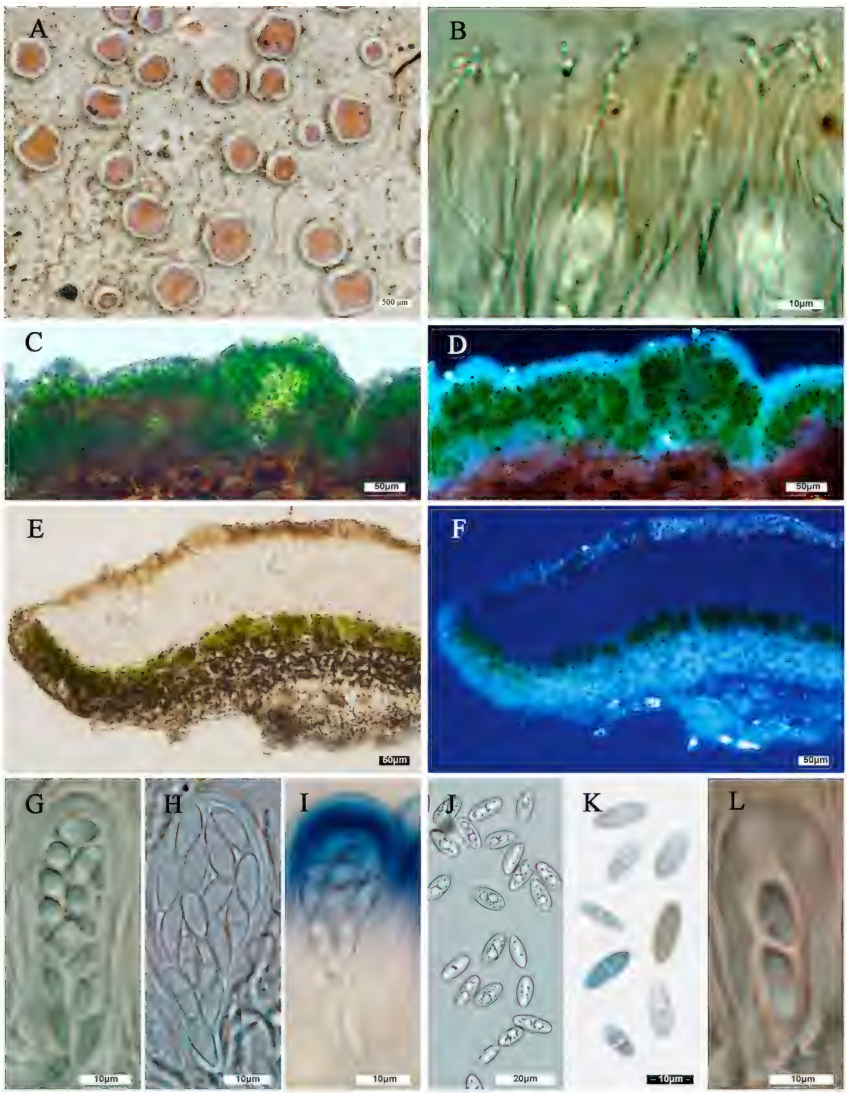

FIG. 1. *Lecanora loekoesii* (20122249, SDNU). A: Thallus; B: Paraphysis; C, E: Anatomy of thallus and apothecia; D, F: Crystals of thallus and apothecia; G–H, J–L: Asci and ascospores; I. Iodine reaction of ascus.

20125751, 20125775. **JILIN**, Helong, Mt. Zengfeng, alt. 1600 m, 19 Aug 2011, Y.L. Cheng 20119440C, 20119440D, 20119643A; alt. 1500 m, 19 Aug 2011, Y.L. Cheng 20120261A, 20119686C, 20119841; alt. 1000 m, 19 Aug 2011, Y.L. Cheng 20119329A; Wangqing, Dadoudigou, alt. 900 m, 23 Jul 2012, H.Y. Wang 20128203, 20128211. **LIAONING**, Kuandian, Mt. Huabo, alt. 1000 m, 16 Aug 2011, Y.D. Wei 20120438C; alt. 900 m, 14

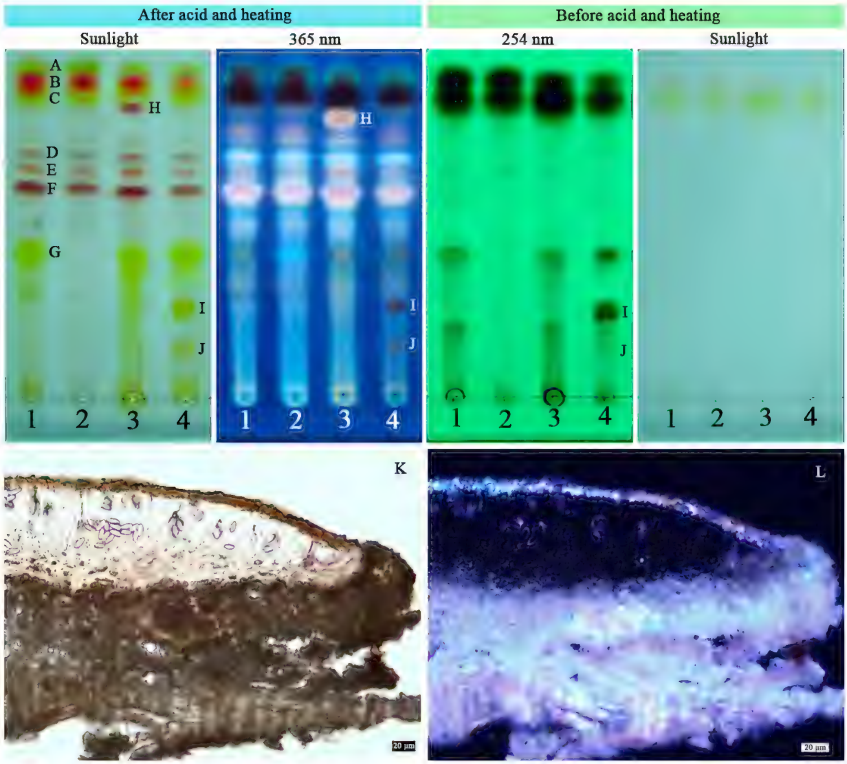

FIG. 2. *Lecanora loekoesii*: TLC results and crystals in holotype apothecium. A: Atranorin; C: Usnic acid; F: Zeorin; G: Norstictic acid; H: Placodiolic acid; I: Stictic acid; B, D, E, J: Unknown substances; K, L: Section and crystals of apothecium of holotype. Chemical type 1: 52 specimens detected, including holotype; Chemical type 2: 14 specimens detected; Chemical type 3: Two specimens detected; Chemical type 4: one specimen detected.

Aug 2011, Y.D. Wei 20120449A, 20120450A: 16 Aug 2011, Y.D. Wei 20120547; alt. 800 m, 16 Aug 2011, Y.L. Cheng 20120514A.

Chemical type 2: **CHINA**. **HEILONGJIANG**, Wuchang, Mt. Datudingzi, alt. 1200 m, 21 Aug 2011, Y.L. Cheng 20122139, 20122261A7, 20122288D; alt. 1100 m, 21 Aug 2011, Y.L. Cheng 20122126B; alt. 900 m, 21 Aug 2011, Y.L. Cheng 20122243B; alt. 800 m, 21 Aug 2011, Y.L. Cheng 20122068A, 20122212A, 20122231B. **JILIN**, Helong, Mt. Zengfeng, alt. 1600 m, 19 Aug 2011, Y.L. Cheng 20119440A; alt. 1500 m, 19 Aug 2011, Y.L. Cheng 20119686B. **HUBEI**, Shennongjia, Mt. Jinhou, alt. 2190 m, 9 Nov 2008, Z.T. Zhao 20101591. **SHAANXI**, Meixian, Mt. Taibai, alt. 2900 m, 17 Jun 2011, Y.L. Cheng 20114386D. **TIBET**, Nyingchi, Lulangzhen, alt. 2500 m, 17 Jul 2011, Y.L. Cheng 20119194, 20119213A.

Chemical type 3: **CHINA**. **HEILONGJIANG**, Tieli, Taoshan national forest park, alt. 800 m, 23 Aug 2011, Y. L. Cheng 20125175; alt. 700 m, 23 Aug 2011, Y. L. Cheng 20125762.

Chemical type 4: CHINA. HEILONGJIANG, Wuchang, Mt. Datudingzi, alt. 1100 m, 21 Aug 2011, Y.L. Cheng 20122106B.

COMMENTS — *Lecanora loekoesii* is similar to *L. strobilinoides* in morphology and anatomy. The latter is the only multispored *Lecanora* species that frequently has 1-septate ascospores. However, *L. strobilinoides* can be readily distinguished by having only usnic acid and zeorin. In addition, *L. loekoesii* ascospores are not truly 1-septate.

Acknowledgements

The project was financially supported by Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province, the National Natural Science Foundation of China (31070010, 31170187), and Science Foundation of Jinan (201202024). We thank Prof. A. Aptroot (ABL Herbarium, Soest, The Netherlands) and Dr. Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for presubmission reviews. We also thank Dr. Alstrup (Institut for Økologisk Botanik, Øster Farimagsgade 2D, DK-1353 København K) for providing the information on *Lecanora polysphaeridia* to us.

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Three new species and one new record of *Lobothallia* from China

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Abstract — A comprehensive analysis of morphological, anatomical, chemical and molecular data indicates the presence of three additional species in the genus *Lobothallia* (*Megasporaceae*, lichenized ascomycetes), which are described here as new to science under the names *L. crassimarginata*, *L. helanensis*, and *L. pruinosa*. *Lobothallia praeradiosa* is newly reported from China.

Key words — lichen, taxonomy

Introduction

The lichen genus *Lobothallia* (Clauzade & Cl. Roux) Hafellner was originally established for four species, *L. alphoplaca, L. melanaspis, L. praeradiosa,* and *L. radiosa* (Hafellner 1991), distinguished from *Aspicilia* by their lobate thallus, small spores, short conidia and chemistry. Recently, five additional species *L. farinosa*, *L. recedens* (Nordin et al. 2010), *L. cernohorskyana*, *L. chadefaudiana,* and *L. cheresina* (Roux 2012) have been included. As a consequence of this extension, *Lobothallia* is characterized by immersed to appressed or constricted-sessile apothecia, asci with an inamyloid tholus (*Aspicilia*-type), unbranched paraphyses, simple, hyaline spores and mainly bacilliform conidia. Lobes are distinct in some species, such as *L. alphoplaca*, *L. melanaspis*, *L. praeradiosa,* and *L. radiosa*, while other species have indistinct lobes, such as *L. farinosa* and *L. recedens*. In phylogenetic studies, *Lobothallia* is the sister group of the other genera within *Megasporaceae* (Nordin et al. 2010).

During our study on *Megasporaceae* of China, we discovered three new species of *Lobothallia,* described here as *L. crassimarginata*, *L. helanensis*, and *L. pruinosa*. *Lobothallia praeradiosa* is reported for the first time from China.

Materials & methods

The specimens studied were collected in Northern and Western China, and are preserved in SDNU (Lichen Section of the Botanical Herbarium, Shandong Normal

Table 1. Specimens used in the phylogenetic analyses. New sequences are in bold.

Species	Origin	Voucher	GenBank No.
Aspicilia cinerea	Sweden	UPS: Nordin 6213	JF703115
A. epiglypta	Sweden	UPS: Nordin 6105	HQ259262
Lobothallia alphoplaca	China, IM	SDNU: 20117646	**JX476025**
	China, IM	SDNU: 20117616	**JX499233**
L. crassimarginata	China, IM	SDNU: 20122565 (holotype)	**JX476026**
	China, IM	SDNU: 20122583	**KC007439**
L. helanensis	China, IM	SDNU: 20122791	**JX476031**
	China, IM	SDNU: 20122517 (holotype)	**JX476030**
L. melanaspis	Sweden	UPS: Nordin 6622	HQ259272
	Norway	UPS: Owe-Larsson 8943a	JF825524
L. praeradiosa	China, XJ	SDNU: 20126683	**JX499229**
	China, XJ	SDNU: 20126355	**JX499230**
	China, XJ	SDNU: 20126314	**JX499232**
	China, XJ	SDNU: 20126613	**JX499234**
L. pruinosa	China, IM	SDNU: 20123630	**JX476027**
	China, IM	SDNU: 20123278 (holotype)	**JX476028**
	China, IM	SDNU: 20123909	**JX499231**
L. radiosa	Sweden	UPS: Nordin 5889	JF703124
L. recedens	Sweden	UPS: Nordin 6035	HQ406807

*Province abbreviations: IM = Inner Mongolia; XJ = Xinjiang.

University). Specimen morphology and anatomy were examined using a dissecting microscope (Olympus SZ51) and a compact light microscope (Olympus CX41). Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent C (Orange et al. 2010). Photos of the thalli were taken using an Olympus SZX16 with DP72.

Sampling: Nuclear ITS1-5.8S-ITS2 rDNA sequences of 18 specimens representing 9 species were used in the molecular study. New sequences were produced from 13 specimens and six sequences were downloaded from GenBank (Tab. 1). *Aspicilia cinerea* and *A. epiglypta* were used as outgroup.

Extractions, PCR amplifications, and sequencing: Total DNA was extracted from the samples using the SanPrep Colum DNA Gel Extraction Kit. Lichen sample vouchers of the new sequences are listed in Table 1. To amplify the ITS1-5.8S-ITS2 regions, the primers ITS1-F (Gardes & Bruns 1993) combined with LR7 (Vilgalys & Hester 1990) or ITS4 combined with ITS5 (White et al. 1990) were used. The PCR ran for 37 cycles (1 min. at 95°C, 30 sec. at 53°C, 30 sec. at 72°C) using Tiangen Taq. Sequencing reactions were carried out by BGI (www.genomics.cn) with an ABI 3730 XL DNA Analyzer.

Phylogenetic analyses: The data set was processed with the Minimum Evolution method (Rzhetsky & Nei 1992). The percentages of replicate trees (1000 replicates) in which the associated taxa clustered together in the bootstrap test (Felsenstein 1985) are shown next to the branches (Fig. 1). The evolutionary distances were computed using

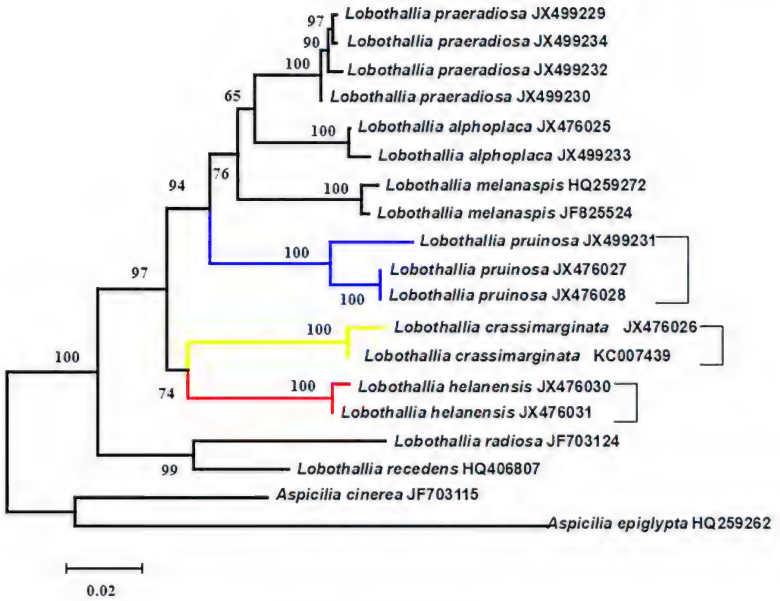

FIGURE 1. The ME tree inferred from ITS data. Bootstrap values greater than 60% (1000 replicates) are shown next to the branches.

the Maximum Composite Likelihood method (Tamura et al. 2004). There were a total of 571 positions in the final dataset. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Results & discussion

New Species

Lobothallia crassimarginata X.R. Kou & Q. Ren, **sp. nov.** FIG. 2

MYCOBANK MB 801753

Differs from all other *Lobothallia* spp. by its persistently prominent apothecial margin and thick thallus.

TYPE: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20122565 (**Holotype**, SDNU; Genbank, JX476026).

ETYMOLOGY: Latin *crassus* (= thick) + *marginatus* (= margined), referring to the apothecial margins.

THALLUS placodioid, up to 2–5 mm thick centrally, tightly adnate, areolate; AREOLES 1–1.5 mm in diam., discrete, rounded, plane to somewhat convex; LOBES 1–2 mm long, 0.5–0.8 mm across, 0.5–0.7 mm or more thick, usually distinctly elongated, radiating and separate, plane to more often strongly convex; UPPER SURFACE ashy gray, sometimes tinted ochraceous or rosy,

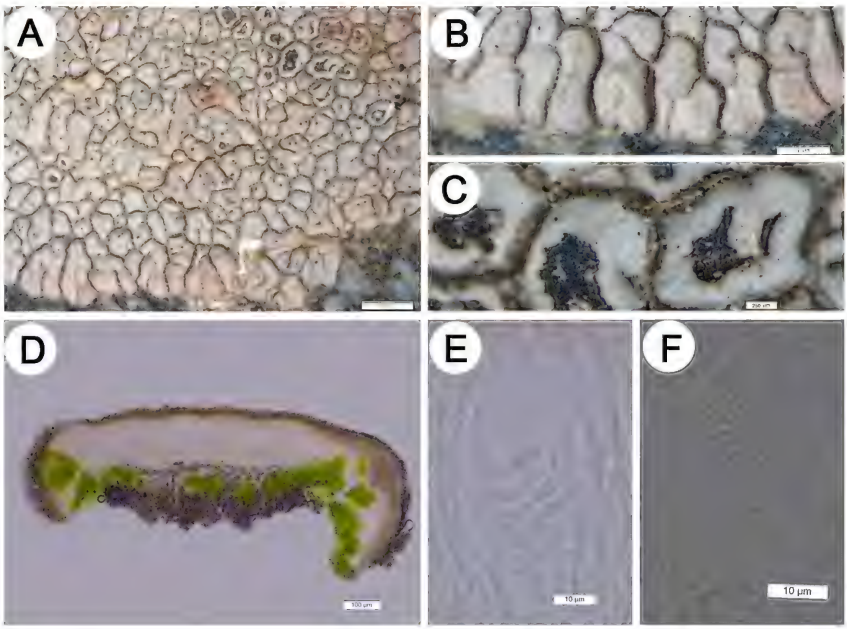

FIGURE 2. *Lobothallia crassimarginata* (Holotype). A. Thallus; B. Lobes; C. Apothecia; D. Apothecial anatomy; E. Ascus and spores; F. Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 250 µm; D = 100 µm; E, F = 10 µm.

usually epruinose, smooth to somewhat wrinkled. APOTHECIA lecanorine, usually solitary, 0.6–1.5 mm in diam., appressed-sessile to distinctly sessile; DISC black, plane or convex, epruinose; MARGIN remaining strongly raised and inflexed, 0.2–0.5 mm wide, concolorous with the thallus; EPIHYMENIUM brown, the pigment fading in K, N+ lightly green; HYMENIUM hyaline, 70–90 µm tall; PARAPHYSES separating in KOH, submoniliform; SUBHYMENIUM and HYPOTHECIUM hyaline, 50–70 µm tall together, with algae layer below; ASCI *Aspicilia*-type, 8-spored; ASCOSPORES hyaline, simple, subglobose to globose, 9–10 × 8–9 µm; CONIDIA bacilliform, 5.4–7.6 × 1–1.2 µm.

SPOT TESTS — medulla K+ yellow then red, C–, I–, P+ orange.

SECONDARY METABOLITES — norstictic, stictic and constictic acids.

SUBSTRATE — calciferous rock.

ADDITIONAL SPECIMENS EXAMINED (all conserved in SDNU) — **CHINA. INNER MONGOLIA**, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20122418B, 20123084B, 20123089C; D.B. Tong 20122513, 20122583 (GenBank, KC007439), 20122518.

COMMENTS — *Lobothallia crassimarginata* differs from the other members of this genus by its persistently prominent apothecial margin and thick thallus. Both *Lobothallia alphoplaca* and *L. praeradiosa* have raised apothecial margins

only when young. Three other *Lobothallia* species with a thick thallus are *L. alphoplaca*, *L. helanensis*, and *L. recedens*. *Lobothallia alphoplaca* differs from *L. crassimarginata* by its intact removable thallus with longer lobes, *L. helanensis* by having a rough thallus, a thin apothecial margin, and lacking secondary metabolites, while *L. recedens* has a pruinose thallus and shorter conidia (3–5 × 1 μm; Smith et al. 2009). In the phylogenetic tree, these four species lie in different clades. Evolutionary distances between *L. crassimarginata* and other three species range from 0.074 to 0.111.

Lobothallia helanensis X.R. Kou & Q. Ren, **sp. nov.** FIG. 3

MycoBank MB 801755

Differs from *Lobothallia recedens* by its thallus having a gray to white-gray upper surface with a brown tinge, and by its larger conidia.

TYPE: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, D.B. Tong 20122517 (**Holotype**, SDNU; Genbank, JX476030).

ETYMOLOGY: Referring to the type locality.

THALLUS crustose, rimose to areolate, gradually thicker towards the central part, sometimes up to 7 mm thick; AREOLES 0.7–1.4(–2) mm in diam., contiguous,

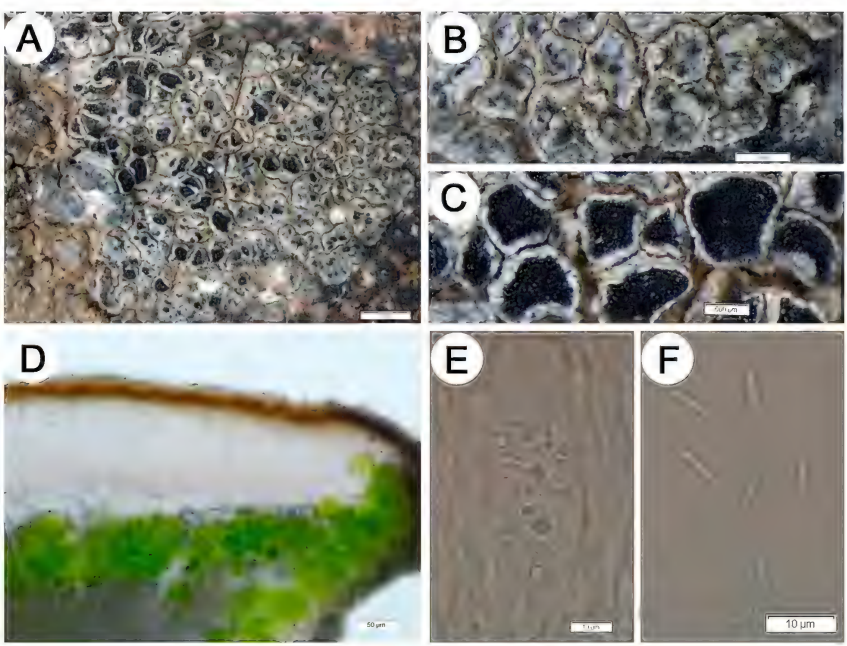

FIGURE 3. *Lobothallia helanensis* (Holotype). A. Thallus; B. Lobes; C. Apothecia; D. Apothecial anatomy; E. Ascus and spores; F. Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 500 μm; D = 50 μm; E, F = 10 μm.

angular to irregular, ±convex; UPPER SURFACE gray to white-gray, with a brown tinge, distinctly rough. APOTHECIA 0.5–1.3(–2) mm in diam., aspicilioid, rounded or angular; thalline margin thin, slightly elevated, sometimes with slightly dentate incision, inner part brown, outer part concolorous with the thallus; DISC black, without pruina; EPIHYMENIUM brown, K+ brown, N+ weakly green; HYMENIUM hyaline, I+ blue, 70–80 µm tall; PARAPHYSES separating in KOH, submoniliform to moniliform; SUBHYMENIUM colourless to weakly brown, I+ blue, ±50 µm tall; HYPOTHECIUM colourless, I+ blue, 30 µm tall, algae continuous or grouped below the hypothecium. ASCI clavate, *Aspicilia*-type, 8-spored; ASCOSPORES hyaline, simple, ellipsoid, 10–12.5 × 5.5–6.5 µm; CONIDIA bacilliform, 5.5–6.4(–8) × 1.2–1.4 µm.

SPOT TESTS — medulla K–, C–, I–, P–.

SECONDARY METABOLITES — none detected by TLC.

SUBSTRATE — calciferous rock.

ADDITIONAL SPECIMENS EXAMINED (all conserved in SDNU) — **CHINA. INNER MONGOLIA**, Mt. Helan, on rock, alt. 1500 m, 17 Aug 2011, D.B. Tong 20122791 (GenBank, JX476031); 19 Aug 2011, H.Y. Wang 20122986, 20123043, 20122780A; P.M. Wang 20123301, 20123308A, 20123709.

COMMENTS — *Lobothallia helanensis* resembles most closely *L. recedens*, which shares a thick thallus and the absence of secondary metabolites but has a white sheen resembling pruina and smaller conidia. *Lobothallia alphoplaca*, another species with a thick thallus, differs by its loosely adnate thallus containing norstictic, constictic, or salazinic acids. In the phylogenetic tree, *Lobothallia helanensis* JX476030 and *Lobothallia helanensis* JX476031 form a clade supported by 99% bootstrap value. These three species lie in different clades. The evolutionary distances between *L. helanensis* and the other two species range from 0.091 to 0.132.

Lobothallia pruinosa X.R. Kou & Q. Ren, **sp. nov.** FIG. 4

MYCOBANK MB 801756

Differs from *Lobothallia recedens* by its thin and pruinose thallus, its more sparse apothecia, and its smaller ascospores.

TYPE: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20123278 (**Holotype**, SDNU; Genbank, JX476028).

ETYMOLOGY: Referring to the pruinose thallus and apothecial discs.

THALLUS placodioid to squamulate, up to 0.5–1.0 mm thick, tightly attached, areolate, at the margin radiate; AREOLES 0.5–1 mm wide, contiguous, plane; LOBES plane, contiguous, confluent, tightly attached, 1–2 mm long, 0.7–1 mm wide, 0.15–0.2 mm thick; UPPER SURFACE usually whitish gray to brownish gray, often pruinose especially at the margins of the areoles. APOTHECIA solitary, round, 0.7–1.2 mm in diam., somewhat higher than the areoles; DISC

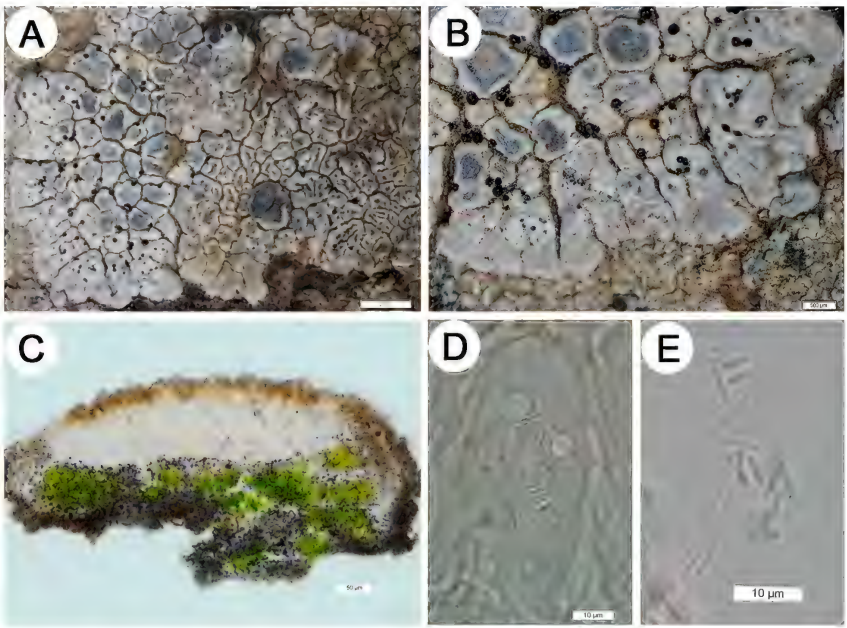

FIGURE 4. *Lobothallia pruinosa* (Holotype). A. Thallus with white pruina; B. Lobes and apothecia; C. Apothecial anatomy; D. Asci and spores; E. Conidia. Scale bars: A = 2 mm; B = 500 µm; C = 50 µm; D, E = 10 µm.

dark brown, usually pruinose; ASCI *Aspicilia*-type, 8-spored; EPIHYMENIUM brown, the pigment fading in K, N+ weakly green; HYMENIUM hyaline, I+ blue, 80–120 µm tall, PARAPHYSES separating in KOH, submoniliform to moniliform; SUBHYMENIUM and HYPOTHECIUM colourless, I+ blue, 50–70 µm tall together, algae continuous or grouped below the hypothecium; ASCOSPORES broadly ellipsoid, 12.5–15 × 8.5–10 µm; CONIDIA bacilliform, 5–7 × 1–1.3 µm.

SPOT TESTS — medulla K+ yellow, C–, I–, P+ orange.

SECONDARY METABOLITES — norstictic and constictic acids.

SUBSTRATE — siliceous rock intermingled with calciferous granules.

ADDITIONAL SPECIMENS EXAMINED (all conserved in SDNU) — **CHINA. INNER MONGOLIA**, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20123091, 20123630 (GenBank, JX476027); 1600 m, 19 Aug 2011, H.Y. Wang 20123575. Mt. Huhebashige, on rock, alt. 1600 m, 16 Aug 2011, H.Y. Wang 20123447B, 20123408; 17 Aug 2011, H.Y. Wang 20123909 (GenBank, JX499231); X.R. Kou 20123915, 20123950; P.M. Wang 20123678.

COMMENTS — Another species with distinct pruina is *L. recedens*, which differs from *L. pruinosa* by its thick thallus, numerous apothecia and somewhat larger spores. The two species lie in different clades. The evolutionary distances between them range from 0.130 to 0.139.

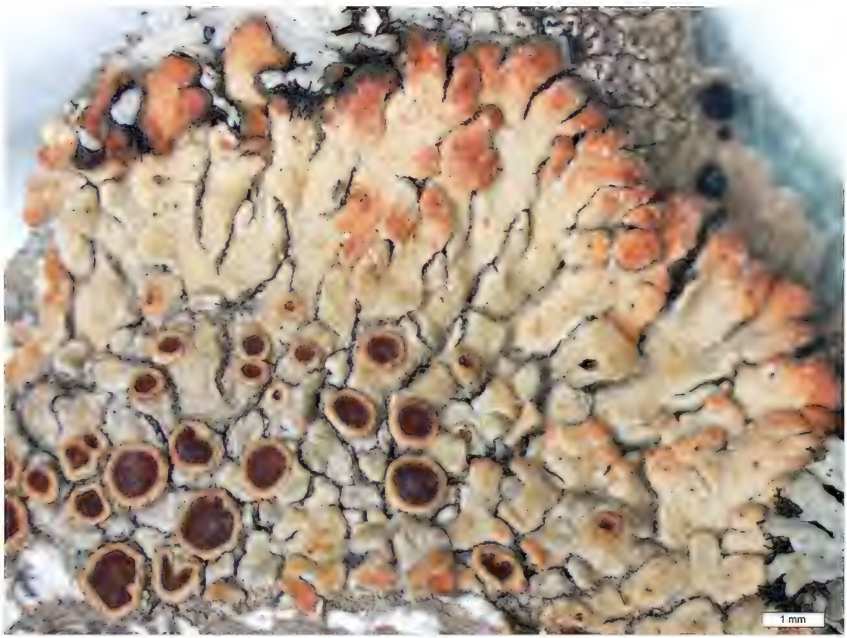

FIGURE 5. *Lobothallia praeradiosa*, 20126613 (SDNU). Scale = 1 mm.

New Record

Lobothallia praeradiosa (Nyl.) Hafellner, Acta Bot. Malac. 16: 138 (1991). FIG. 5

THALLUS placodioid, up to 0.5–1.0 mm thick, tightly attached, areolate; AREOLES 0.5–1 mm wide, contiguous, elongate to irregular, plane to convex; LOBES contiguous, confluent, plane, broad, sometimes imbricate, loosely attached, 3–6 mm long, 0.5–1.5 mm wide, 0.2–0.5 mm thick; UPPER SURFACE usually green gray to orange brown, often radiate. APOTHECIA solitary to numerous, round, 0.5–1.5 mm in diam., adnate, somewhat higher than other areoles; DISC: dark brown, usually epruinose, ASCI *Aspicilia*-type, 8-spored; EPIHYMENIUM brown, K+ brown, N–; HYMENIUM hyaline, I+ blue, 75–100 μm tall, PARAPHYSES separating in KOH, submoniliform; SUBHYMENIUM and HYPOTHECIUM colourless, I+ blue, 40–50 μm tall together; algae continuous or grouped below the hypothecium; ASCOSPORES ellipsoid, 12.5–15 × 5–7.5 μm.

SPOT TESTS — medulla K+ yellow, C–, I–, P+ orange or yellow.

SECONDARY METABOLITES — norstictic acid.

SUBSTRATE — siliceous rock.

ADDITIONAL SPECIMENS EXAMINED (all conserved in SDNU) — **CHINA. XINJIANG**, Mt. Tian, on rock, alt. 1910 m, 23 Aug 2011, Z.L. Huang 20126613 (GenBank, JX499234), Z.L. Huang 20126355 (GenBank, JX499230); L. Li 20126314 (GenBank, JX499232); Mt. Nan, on rock, alt. 1900 m, 28 Aug 2011, Z.L. Huang 20126683 (GenBank, JX499229).

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MYCOTAXON

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A new halotolerant species of *Alternaria* from Qinghai-Tibet Plateau, China

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Abstract — A new halotolerant species of *Alternaria, A. xiaochaidanensis,* is described and illustrated. The specimen was collected from a salt lake on Qinghai-Tibet Plateau. The type specimen is deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP).

Key words — taxonomy, saline, extremophiles

Introduction

Salt tolerant fungi have evolved special physiological mechanisms and metabolic products to survive in the extreme environment of salt-water lakes. In recent decades, more attention has been paid to salt tolerant fungi – both halotolerant and halophilic fungi. Cronin & Post (1977) described the first halophilic filamentous fungus, *Cladosporium* sp., from a hypersaline lake in the Great Salt Lake, USA. To date, more than 80 halotolerant fungal species and six halophilic fungi have been reported (Gunde-Cimerman et al. 2005, 2009).

Qinghai-Tibet Plateau region has the world's highest altitude salt lake. It is a potentially huge treasure trove of genetic resources with its diversity of water chemistry characteristics and different biotic and abiotic anti–positive gene. Thus, it is important to study microorganisms of Qinghai-Tibet Plateau, especially halophilic and halotolerant microorganisms. Here, we describe a new species of *Alternaria* that was isolated from Xiaochaidan, a salt lake on Qinghai-Tibet Plateau. This is the first report of a halotolerant *Alternaria* species.

Alternaria xiaochaidanensis F. Wang, A.N. Li, D.M. Dai, X.X. Xu & D.C. Li **sp. nov.**
MycoBank MB 800882 Fig.1

Differs from *Alternaria chlamydospora* and *A. mouchaccae* by its larger usually beaked conidia and its different conidial shape.

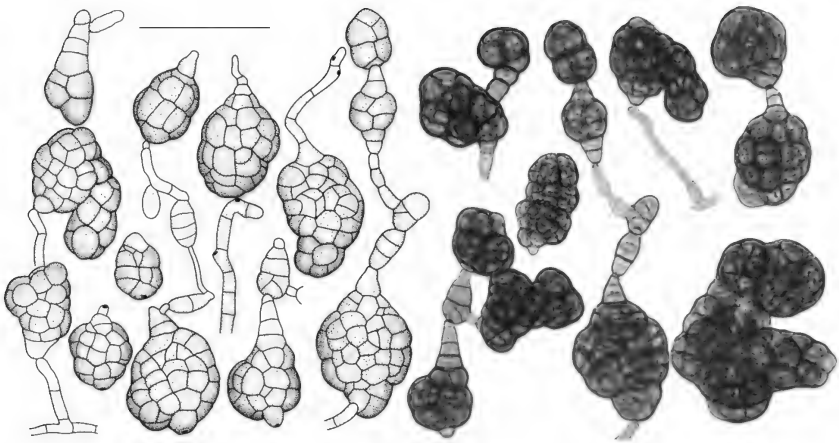

Fig. 1. *Alternaria xiaochaidanensis* (ex holotype).
Conidia, conidiophores and conidiogenous cells. Left: drawings. Right: photomicrographs. (Bar = 50 μm).

Type: China, Qinghai, Xiaochaidan, from a lake soil, altitude 2800–2840 m, July 2011, F. Wang (**Holotype**, HSAUP II 011501; GenBank, JX235958).

Etymology: in reference to the type locality.

Alternaria xiaochaidanensis grows rapidly, colonies on potato carrot agar at 28°C reach 6.5–7.5 cm diam. in 7 days, effuse, circular, pale brown; reverse fuscous-black. Mycelium superficial or immersed; hyphae hyaline, grey–white. Conidiophores either solitary, not branched, 28–54 (–68) × 3–5 μm, or arising as lateral branches from superficial hyphae and up to 170 μm long. Conidia produced from conidiophores or directly from hyphae, solitary or in short chains of 2-5, rostrate or non–rostrate, 13–74 × 6–61μm. Juvenile conidia ovoid or obclavate, pale brown; mature conidia symmetrical or asymmetrical, irregular in shape and size, brown, smooth, walls thickened, with transverse , longitudinal and oblique septa, often constricted at septa.

Comments –This species may be confused with *A. mouchaccae* (Simmons 1981) and *A. chlamydospora* (Mouchacca 1973). However, conidia of those species are smaller: *A. mouchaccae* (25–40(–50) × 12–20(–22) μm) and *A. chlamydospora* (26–70 × 8–48 μm). Although sometimes found in chains of 2–5, conidia in *A. xiaochaidanensis* are usually single; in *A. chlamydospora* conidia are often in chains of 5–8 while conidia of *A. mouchaccae* are rarely in chains. In contrast to *A. xiaochaidanensis,* conidia of *A. mouchaccae* and *A. chlamydospora* have no beak. A conspicuous characteristic of the new species is that mature conidia are symmetrical or asymmetrical and have specific shapes that obviously differ from those of *A. chlamydospora.* Conidiophores of

A. xiaochaidanensis are longer than those of *A. chlamydospora* (15–18 × 3 μm, up to 150 μm) and shorter than those of *A. mouchaccae* (40–50 × 3–4 μm).

A BLAST search comparison of its sequence in GenBank places *A. xiaochaidanensis* into *Alternaria* but with no previously described species close to it.

Gunde-Cimerman et al. (2005, 2009) characterized fungi that can grow in vitro at 3 M salt concentration and are regularly isolated from global environments at salinities above 1.7 M as halophilic, whereas isolates that can grow in vitro sporadically at 3 M NaCl are considered halotolerant. Species isolated above 1.7 M NaCl grow regularly on PDA but slowly at 3 M. According to this definition, the authors consider *Alternaria xiaochaidanensis* a halotolerant fungus.

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The authors are grateful to Dr. Eric McKenzie, Prof. Y.L. Guo, and Dr. Shaun Pennycook for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (31000007), China Postdoctoral Science Foundation (20100471565) and the Chinese National Special Fund of Sea Renewable Energy Sources (SDME2011SW01).

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Additions to the smut fungi of Pakistan. 2

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Abstract — Two species of smut fungi, *Sporisorium lingii* on *Themeda anathera* and *Urocystis oryzopsidis* on *Piptatherum microcarpum,* are reported for the first time from Pakistan. Both smut fungi were found on new host plants. *Chrysopogon serrulatus* is recorded from Pakistan as a new host of *Anthracocystis chrysopogonis.*

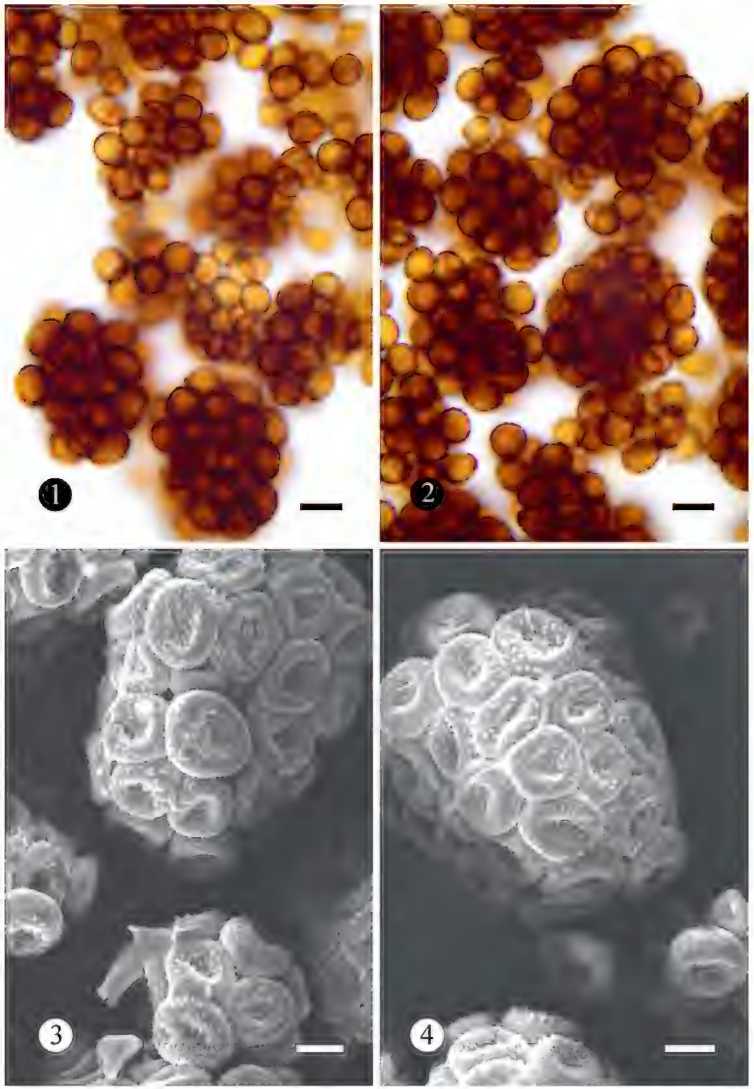
Key words — *Poaceae,* taxonomy, *Ustilaginomycetes*

Introduction

Three smut fungi of poaceous hosts are described in this third contribution to the smut fungi of Pakistan based on material collected by M. Fiaz in 2009–10, in Mansehra District (Khyber Pakhtunkhwa Province) (Denchev et al. 2012, Fiaz et al. 2013). Two species, both on new hosts, *Sporisorium lingii* on *Themeda anathera* and *Urocystis oryzopsidis* on *Piptatherum microcarpum,* are reported for the first time from Pakistan. *Anthracocystis chrysopogonis* is recorded on a new host, *Chrysopogon serrulatus.*

Material & methods

Dried specimens from the Hazara University Herbarium (HUP), Pakistan were examined under light (LM) and scanning electron (SEM) microscopes. For LM observations, spores were mounted in lactophenol solution on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. Spore measurements are given in the form: min–max (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold with an ion sputter. The surface structure of spores was observed at 10 kV and photographed with a JEOL SM-6390 scanning electron microscope.



Figs 1–4. *Anthracocystis chrysopogonis* on *Chrysopogon serrulatus* (HUP 307). Spores in LM and SEM. Scale bars: 1, 2 = 10 µm; 3, 4 = 5 µm.

Taxonomy

Anthracocystis chrysopogonis (Vánky) McTaggart & R.G. Shivas, Persoonia 29: 120, 2012. Figs 1–4

≡ *Sporisorium chrysopogonis* Vánky, Mycotaxon 18: 327, 1983.

Sori destroying the whole inflorescence, partly hidden by the leaf sheath, up to 22 mm in length and 2 mm in width; initially covered by an yellowish brown

peridium which later ruptures from its apex exposing semi-agglutinated, dark reddish brown mass of spore balls and numerous filiform columellae. Spore balls subglobose, broadly ellipsoidal, ovoid or slightly irregular, 23–66 × 20–45 µm, dark to middle reddish brown, composed of 8 to tens of spores that separate by pressure. Single sterile cells very rarely present; subglobose, broadly ellipsoidal, slightly irregular, sometimes collapsed, 8.5–13 × 8–12 µm, hyaline; wall 1.2–2 µm thick, smooth. Spores dimorphic, globose, subglobose, broadly ellipsoidal, ovoid or slightly irregular. Outer spores 8–12(–13) × 7–10 (10.0 ± 0.9 × 8.4 ± 0.6) µm (n = 50), middle reddish brown; wall uneven, 0.7–1.3 µm thick, smooth on the contact surface, verruculose-echinulate on the free surface, spore profile slightly affected. In SEM verruculose-echinulate, finely punctate between the main ornaments. Inner spores (7–)7.5–11 × 6.5–9 (8.6 ± 0.8 × 7.6 ± 0.5) µm (n = 50), light yellowish brown; wall evenly thickened, 0.4–0.7 µm thick, smooth to very finely punctate.

Specimen examined — On *Chrysopogon serrulatus* Trin.: **PAKISTAN, Khyber Pakhtunkhwa Province,** Mansehra District, Chulandrian (Oghi), August 2010, leg. M. Fiaz, no. FS–23 (HUP 307).

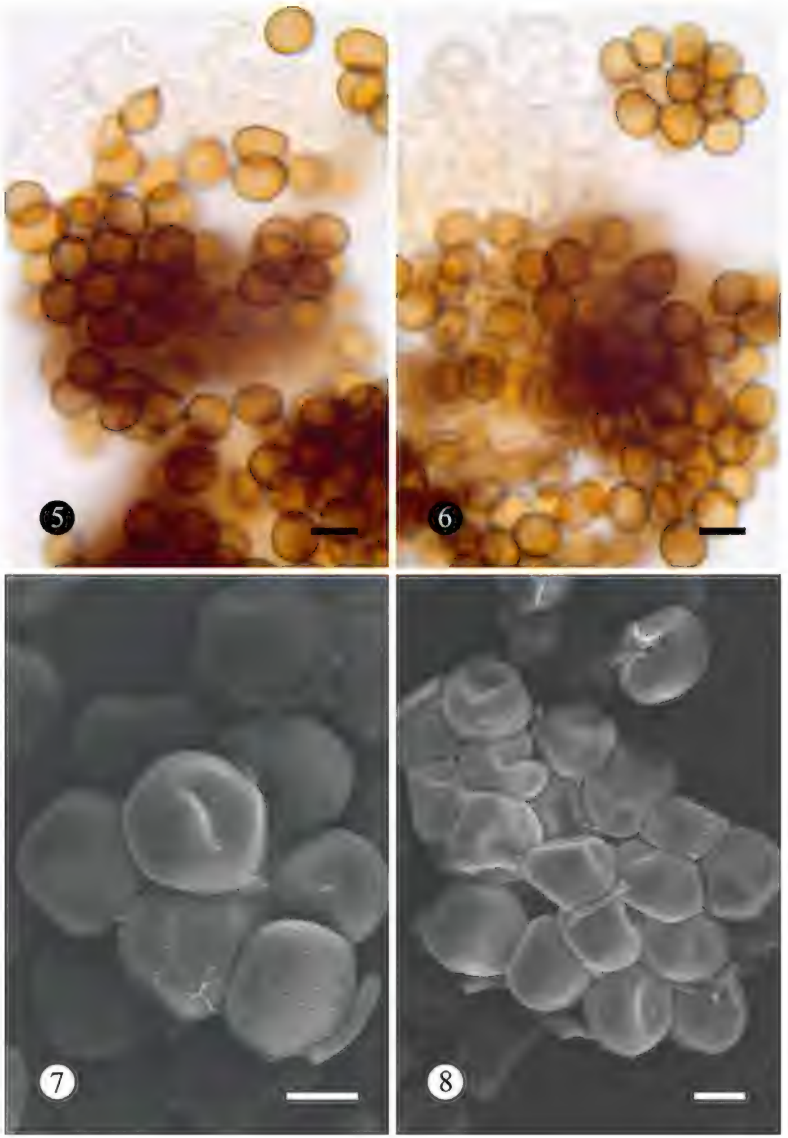
Distribution — This species has been previously known on *Chrysopogon fulvus* (Spreng.) Chiov. (= *C. montanus* Trin.) and *Chrysopogon* sp. from Sri Lanka and Pakistan (Vánky 2005, 2011). *Chrysopogon serrulatus* is a new host record for this smut fungus.

Comments — The spore balls of this specimen are a little more loose compared with those of the isotype (on *Chrysopogon fulvus,* Sri Lanka, North Central Province, Polonnaruwa Distr., Habarane, alt. 250 m, 18 March 1974, K. Vánky, in Vánky, Ustilag. exs., no. 407, SOMF), studied by us.

Sporisorium lingii Vánky, Mycotaxon 51: 160, 1994. Figs 5–8

Sori in all of the spikelets of the raceme, all racemes of an inflorescence affected, 5–12 × 1–2 mm, cylindrical, initially covered by an yellowish brown peridium that soon flakes away exposing a single, branched columella, surrounded by a dark brown mass of readily disintegrating spore balls, spores, and sterile cells. Spore balls loose, middle to dark reddish brown, composed of tens to hundreds of spores. Sterile cells in irregular groups, subglobose to irregular, often collapsed, 9–19 × 8.5–18 µm, hyaline to subhyaline; wall 0.9–1.9 µm thick, smooth. Spores globose, subglobose, broadly ellipsoidal or slightly irregular, 8.5–12.5 × 7.5–11 (10.3 ± 0.7 × 9.3 ± 0.6) µm (n = 100), middle reddish brown; wall evenly thickened, 0.6–0.9 µm thick, smooth to very finely punctate. In SEM very finely echinulate, densely punctate between the spinules.

Specimen examined — On *Themeda anathera* (Nees ex Steud.) Hack.: **PAKISTAN, Khyber Pakhtunkhwa Province,** Mansehra District, Oghi, Khabbal Paien, August 2010, leg. M. Fiaz, no. FS–21 (HUP 309).



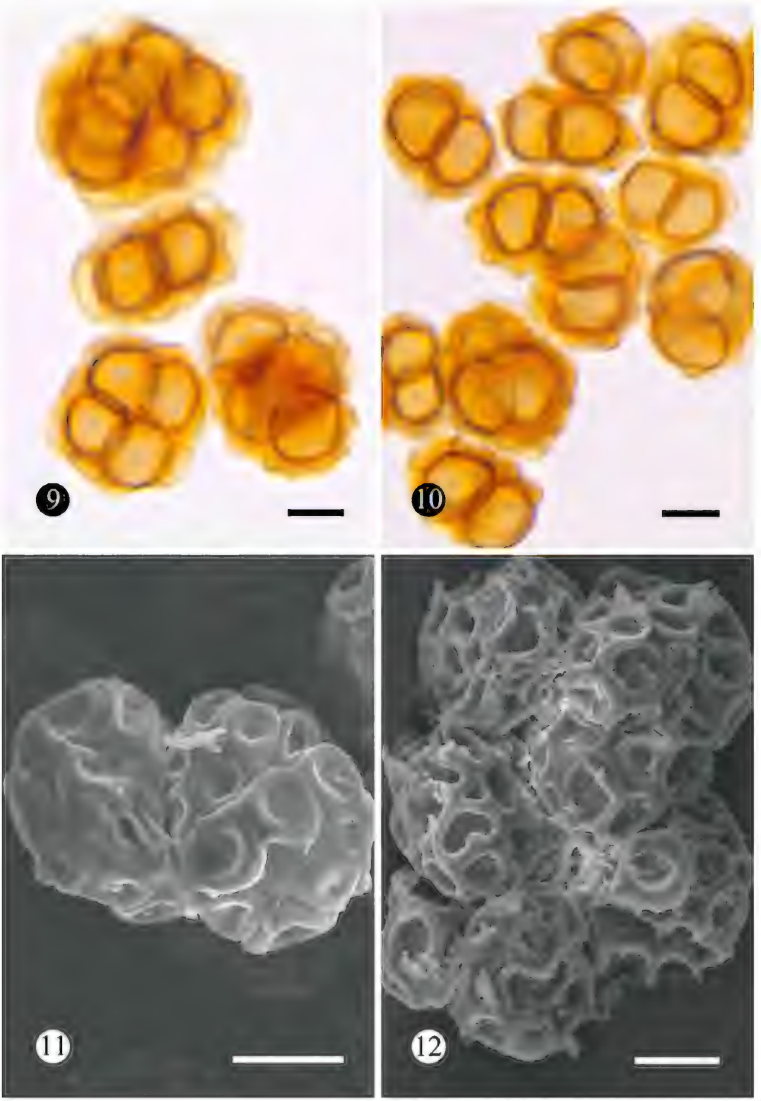
Figs 5–8. *Sporisorium lingii* on *Themeda anathera* (HUP 309).
Spores and sterile cells in LM and SEM. Scale bars: 5, 6 = 10 µm; 7, 8 = 5 µm.

Distribution — This species has been previously known on *Themeda triandra* Forssk. and *Themeda* sp. from S. Asia and Australia (Vánky 2011). *Themeda anathera* is a new host record for this species.

Urocystis oryzopsidis Padwick & Azmatullah, Mycological Papers 10: 1, 1944.

Figs 9–12

Sori in leaves, as long striae between the veins, at first lead-coloured and covered by the epidermis which later ruptures and disclosing the spore balls. Spore balls mass powdery, dark reddish brown. Spore balls subglobose,



Figs 9–12. *Urocystis oryzopsidis* on *Piptatherum microcarpum* (HUP 315). Spore balls in LM and SEM. Scale bars: 9, 10 = 10 μm; 11, 12 = 5 μm.

broadly ellipsoidal, ellipsoidal or irregular, composed of 1–5(–6) central spores [1 = 6.1%, 2 = 27.9%, 3 = 42.3%, 4 = 16.9%, 5 = 5.8%, 6 = 1.0%; n = 409], usually completely invested by a layer of sterile cells; 15–25.5 × 15–20.5 µm [with 1 spore], 17.5–31 × 15.5–20.5 µm [with 2 spores], 21–34.5 × 17–27.5 µm [with 3 spores], 26–39.5 × 18.5–32.5 µm [with 4 spores]. Sterile cells variable in shape, often collapsed, 6–16 µm long, light yellowish brown; wall 0.8–1.5 µm thick, smooth. Spores subglobose, broadly ellipsoidal, ovoid, slightly irregular, sometimes flattened on the contact sides, 10.5–17(–18) × 8–13 (13.7±1.6 × 10.8±1.0) µm (n = 50), medium reddish brown; wall 0.8–1.3 µm thick, smooth.

Specimen examined — On *Piptatherum microcarpum* (Pilg.) Tzvelev (= *P. vicarium* (Grigorj.) Roshev.): **PAKISTAN, Khyber Pakhtunkhwa Province,** Mansehra District, Jaba, 3 September 2009, leg. M. Fiaz, no. FS–12 (HUP 315).

Distribution — This smut fungus has been previously known only from the type locality (India, Kashmir) on *Piptatherum munroi* (Stapf ex Hook. f.) Mez (as *Oryzopsis munroi*).

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MYCOTAXON

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Yunchangia, a new genus of smut fungi (*Ustilaginaceae*) from China

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Abstract —*Yunchangia puccinelliae* gen. et sp. nov. on *Puccinellia glauca*, is described. The fungus was collected from Xinjiang Uygur Autonomous Region, China.

Key words —*Ustilaginomycetidae*, *Ustilaginales*, taxonomy

Introduction

The Taklimakan desert located in the southern Xinjiang Uygur Autonomous Region is the second largest shifting sand desert in the world, covering 337,600 km^2. During a mycological expedition to the margin of the western desert in 2010, a smut species on *Puccinellia glauca* was collected. The sorus of the smut causes a short, longitudinal leaf streak. It is covered by the epidermis and simultaneously contains fungal hyphae and single ustilospores. The fungal hyphae lie longitudinally along the sides of the sorus. Attempts to germinate ustilospores from *Puccinellia glauca* were carried out several times at 24°C on PDA (potato dextrose agar), but failed. To date 269 species of smut fungi have been reported in China (Guo 2000, 2011, He et al. 2011).

Material & methods

Ustilospores and hyphae were mounted in lactophenol and examined by light microscopy (LM), after heating to boiling point. For scanning electron microscopy (SEM), dried ustilospores and hyphae were dusted onto double-sided adhesive tape, fixed on specimen stubs, sputter coated with gold, ca. 20 nm thick, and studied with a FEI Quanta 200 electron microscope.

Taxonomy

Yunchangia L. Guo & B. Xu, **gen. nov.**

Fungal Name FN570049

Differs from *Ustilago* by its sorus simultaneously containing fungal hyphae and ustilospores.

Type species: *Yunchangia puccinelliae* L. Guo & B. Xu

Etymology: The genus is dedicated to Professor Wang Yunchang, an outstanding Chinese mycologist on taxonomy of smut and rust fungi.

Sori on host plant in the *Poaceae* forming short streaks mainly on leaves, covered by the epidermis. Ustilospores single, pigmented. The fungal hyphae lie longitudinally along the sides of the sorus. Hyphae are composed of pigmented cells.

Comments: *Yunchangia* is similar to *Ustilago* (Pers.) Roussel, which differs by absence of fungal hyphae in the sorus (Vánky 2002). Its characters demonstrate that the genus *Yunchangia* is a member of the family *Ustilaginaceae.*

Yunchangia puccinelliae L. Guo & B. Xu, **sp. nov.** Figs 1–6

Fungal Name FN 570050

Differs from *Ustilago striiformis* by its slightly larger ustilospores and with fungal hyphae in sorus.

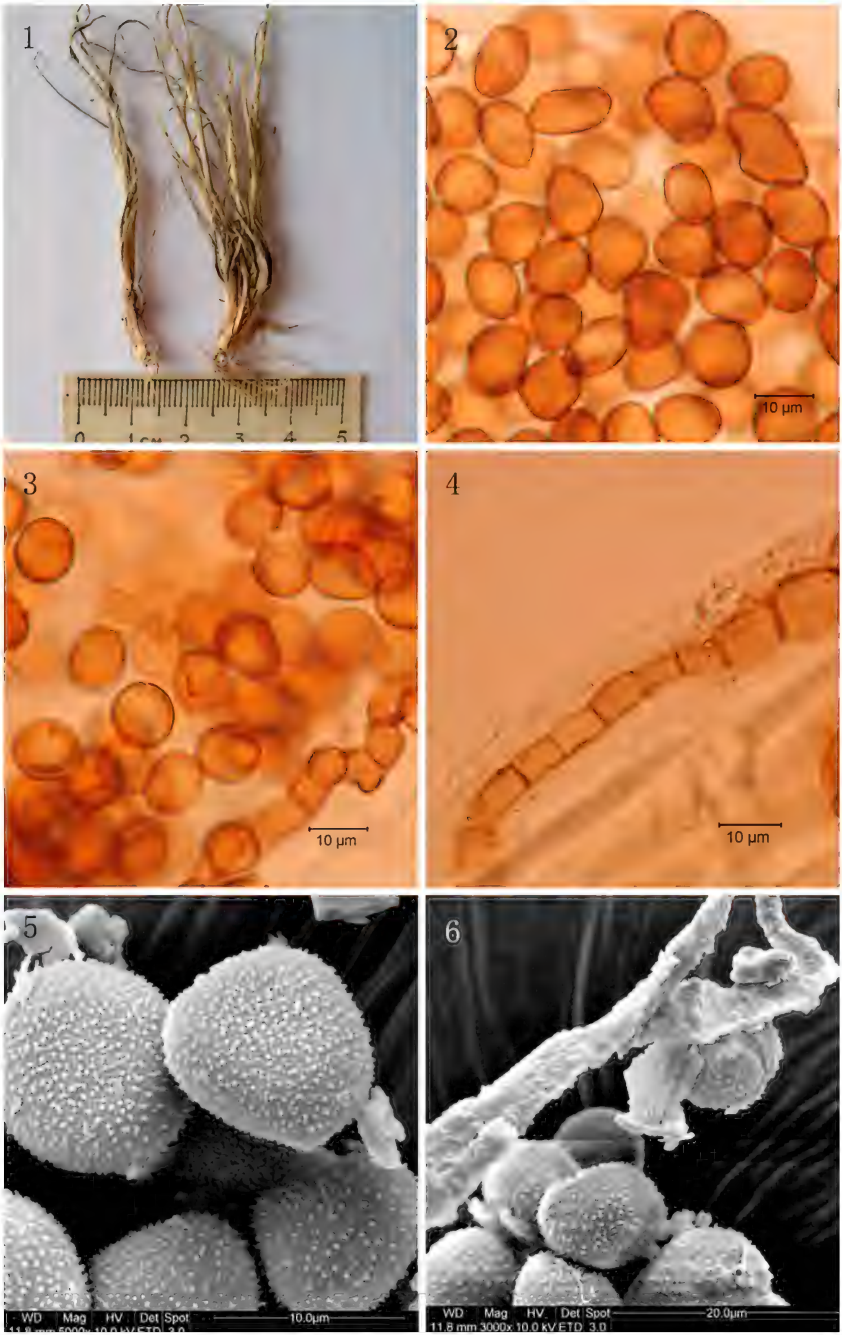
Type: China, Xinjiang, Wuqia, Baykurt, alt. 2600 m, on *Puccinellia glauca* (Regel) V.I.Krecz. ex Drobow (*Poaceae*), 30.VIII.2010, Y.F. Zhu & L. Guo 231 (HMAS 243467, **holotype;** HMUT 1228, **isotype**).

Etymology: The epithet refers to the host plant genus.

Sori in leaves as short streaks, sparsely in pedicels and inflorescences, 0.5–3.5 mm long, covered by the epidermis which later ruptures exposing powdery, dark brown spore mass. Ustilospores subglobose, ellipsoidal, ovoid or irregular, 9–19 × 7–12.5 μm, reddish brown; wall 0.5–0.8 μm thick, echinulate as seen by LM, finely verruculose between the spines as seen by SEM. Fungal hyphae are composed of cells; cells cylindrical, ovoid or irregular, 5–10.5 × 4–8 μm, reddish brown; wall 0.5–0.8 μm thick, smooth as seen by LM, rough or verruculose as seen by SEM.

Comments: *Yunchangia puccinelliae* is similar to *Ustilago striiformis* (Westend.) Niessl, which differs by its slightly smaller ustilospores [9–15(–16) × 8–12(–13) μm] and the absence of fungal hyphae in the sorus (Vánky 1994).

Figs. 1–6. *Yunchangia puccinelliae* (HMAS 243467, holotype). 1. Sori in leaves. 2–4. Ustilospores and fungal hyphae (LM). 5–6. Ustilospores and fungal hyphae (SEM).


1
2
10 μm
3
10 μm
4
10 μm
5
WD Mag HV Det Spot
10.0μm
6
WD Mag HV Det Spot
11.8 mm 3000x 10.0 kV ETD 3.0
20.0μm

Acknowledgements

The authors would like to express their deep thanks to Dr. K. Vánky (Tübingen, Germany) for suggestion to describe the new genus, to Drs. Eric H.C. McKenzie (Auckland, New Zealand) and Cvetomir M. Denchev (Sofia, Bulgaria) for serving as pre-submission reviewers, to Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review, and to Dr. Wenli Chen (Institute of Botany, Chinese Academy of Sciences) for identifying the host plant. This study was supported by the National Natural Science Foundation of China (No. 30960019).

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MYCOTAXON

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Peltaster fructicola, a newly recorded species from China associated with sooty blotch and flyspeck

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Abstract — *Peltaster fructicola* is first reported and described from China. This fungus was isolated from peels of crabapple and hawthorn collected from Liaoning and Shaanxi Provinces and exhibited the punctate mycelial type of sooty blotch and flyspeck on both hosts. Our isolates were identified based on morphological characteristics and DNA sequence analysis.

Key words — *Rosaceae,* taxonomy, morphology, phylogeny

Introduction

Sooty blotch and flyspeck (SBFS) is a commonly occurring disease complex in humid and temperate regions worldwide, causing blemishes on apple, pear, banana, and other cultivated crops that result in economic losses (Williamson & Sutton 2000, Batzer et al. 2005, Gleason et al. 2011). The SBFS fungi colonize the waxy cuticle of plants and display various mycelial types. The term "sooty blotch" refers to colonies on hosts that consist of a dark mycelial mat with or without sclerotium-like bodies, whereas "flyspeck" denotes a cluster of black dots without a visible mycelial mat. "Sooty blotch" was attributed to a single fungal species for more than 75 years (Colby 1920, Williamson & Sutton 2000) until the late 1990s, when Johnson et al. (1996, 1997) found three "sooty blotch" species based on morphological examination of sooty blotch isolates from apples in USA. One newly described species from that work was *Peltaster fructicola* (Johnson et al. 1996).

During a recent survey of SBFS complex on fruit of rosaceous hosts in China, crabapples and hawthorns were found to display colonies of SBFS. Based on analysis of morphology and DNA sequences of nuclear ribosomal ITS region, our isolates were identified as *P. fructicola.*

Materials & methods

Isolates and morphology

Crabapple *(Malus ×micromalus)* and hawthorn *(Crataegus pinnatifida)* fruit with SBFS signs were collected from Liaoning and Shaanxi Provinces. Two isolates were obtained from hawthorns in Liaoning and Shaanxi Provinces, and three from crabapples in Liaoning Province. Peels displaying SBFS were examined, photographed and preserved. Thalli were transferred directly from peels to potato-dextrose agar (PDA) and cultured at 25°C in the dark (Sun et al. 2003). After one month, pure isolates were transferred to PDA and water agar (WA, 2% agar) for morphological examination. Colony descriptions were based on cultures on PDA after 3 weeks in the dark at 25°C. Morphological descriptions were made on WA and synthetic nutrient-poor agar (SNA) after 3 weeks using the method of Zhang et al. (2009). Specimens and representative dried culture were deposited in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from fungal mycelium according to the protocol of Barnes et al. (2001). The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The PCR conditions followed the methods of Zhuang et al. (2010). PCR products were sequenced by Sangon Biotech (Shanghai), China.

Sequence alignment and phylogenetic analysis

Sequences generated in this study were added to other sequences of *Peltaster* spp., as well as the outgroup species *Mycosphaerella lateralis* and *Schizothyrium pomi* obtained from GenBank. Preliminary alignments were performed using CLUSTAL X (Thompson et al. 1997), and then manually adjusted using BioEdit v. 5.0.9.1 (Hall 1999). Phylogenetic analysis of aligned sequences was performed using PAUP v. 4.0b 10 (Swofford 2003). The heuristic search option was 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. The robustness of clades and internal branches was evaluated by 1000 bootstrap replications. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Sequences generated in this study were deposited in GenBank. Alignment and the representative tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13540).

Results

Phylogenetic analysis

The manually adjusted ITS alignment contained 27 sequences (including the outgroup sequences). Of the 544 characters used for the phylogenetic analysis, 153 were parsimony-informative, 95 were variable and parsimony-uninformative, and 296 were constant. The first 100 equally most parsimonious trees were obtained from the parsimonious analysis, and the first of which is shown in Fig. 1 (TL = 403 steps, CI = 0.8734, RI = 0.9373, RC = 0.8187). The *Peltaster* species formed a strongly supported clade with a bootstrap value of

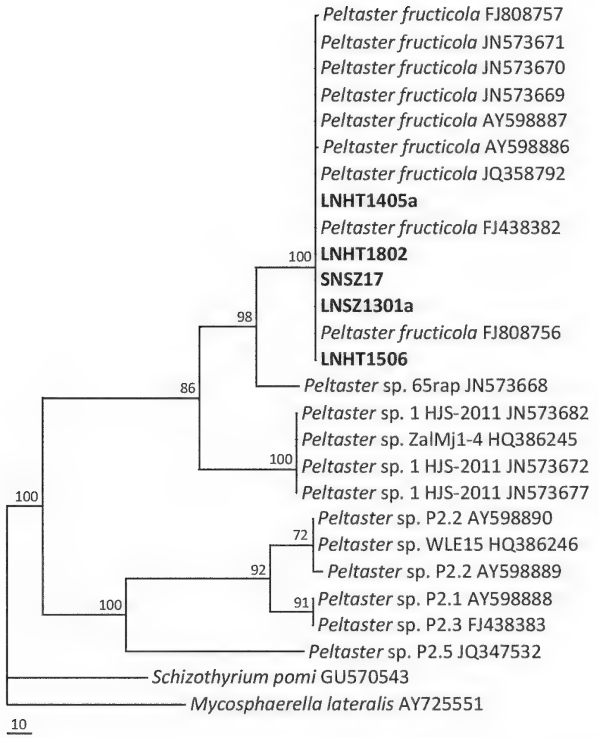

FIG. 1. One of 100 equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the ITS sequence alignment. The bootstrap support values (50%) based on 1000 replicates are shown at the nodes. The tree was rooted to *Mycosphaerella lateralis* and *Schizothyrium pomi*. The scale bar shows 10 changes. Strains investigated in this paper are presented in bold.

100%, which further divided into two subclades. Our five strains clustered together with *Peltaster fructicola* within one of the subclades with a high bootstrap value of 100%, indicating that they might represent the same species (FIG. 1).

Taxonomy

Peltaster fructicola Eric M. Johnson, T.B. Sutton & Hodges,
Mycologia 88(1): 120, 1996 FIG. 2

HYPHAE smooth, septate, branched, hyaline and turning to pale brown as culture aged, 1.5–2.0 µm wide. CONIDIOPHORES absent. CONIDIOGENOUS CELLS hyaline, monoblastic, intercalary, formed directly on undifferentiated hyphae. Conidiogenous loci inconspicuous, stub-shaped, concolorous with conidiogenous cells. CONIDIA solitary, unicellular, hyaline, elliptic to ovoidal, produced in basipetal order from the conidiogenous loci and aggregated in

masses near the conidiogenous cells, 4–6.5(–7) × 1.5–2.5 µm. Microcyclic conidiation observed in culture, with expansion and germination of conidia. Conidia on WA slightly smaller in size than those on SNA, 4–6(–7) × 1.5–2.0 µm.

Cultural characteristics — On PDA erumpent, compact, with smooth, crenate margin and sparse to no aerial mycelium, surface strongly folded, outer zone light gray, center greenish gray to olivaceous-gray, reverse black with yellow masses of conidia at the bottom of the colony; reaching (13–)14(–16) mm diam after 21 d at 25°C in the dark.

On peels of crabapple fruit, showing punctate mycelial type, visible mycelial mat with dull brown, flattened, circular or irregular sclerotium-like bodies, 68–145 µm diam. On peels of hawthorn fruit similar, sclerotium-like bodies 62–223 µm diam.

Specimens examined (all conserved in HMUABO): **China, Liaoning Province**: Huludao City, Suizhong County, 40°19′32″N 120°17′28″E, on fruit surface of crabapple (*Malus* ×*micromalus* Makino), Sept. 2010, Zhuang JL LNHT1506 (GenBank JX961608), LNHT1802 (GenBank JX961609), LNHT1405a (GenBank JX961610); on fruit surface of hawthorn (*Crataegus pinnatifida* **Bunge**), Sept. 2010, Zhuang JL LNSZ1301a (GenBank JX961607). **Shaanxi Province**: Shangluo City, Shangnan County, on fruit surface of hawthorn (*Crataegus pinnatifida*), 21 Oct. 2011, Chen C & Dang JL SNSZ17 (GenBank JX961611).

Discussion

The genus *Peltaster* was established by Sydow & Sydow in 1917; the type species is *Peltaster hedyotidis* Syd. & P. Syd. Sutton and his colleagues described *P. fructicola* (Johnson et al. 1996) as one of the pathogens of sooty blotch and later associated it with punctate mycelial type on apple (Williamson et al. 2004). Since then, this fungus has been found widely in the eastern and midwestern United States (Johnson et al. 1997, Batzer et al. 2005, Díaz Arias et al. 2010). As the etiology of SBFS is studied worldwide, *P. fructicola* has been reported to cause sooty blotch in many other countries and regions, including Serbia, Montenegro, and Poland (Ivanović et al. 2010, Mirzwa-Mróz & Wińska-Krysiak 2011), indicating that *P. fructicola* is among the most prevalent SBFS species.

Based on phylogenetic analysis of the ITS region and morphological comparison, we identified our isolates as *Peltaster fructicola*, a newly recorded species for China. To our knowledge, this is also the first report of SBFS on crabapple and hawthorn caused by *P. fructicola.*

Acknowledgments

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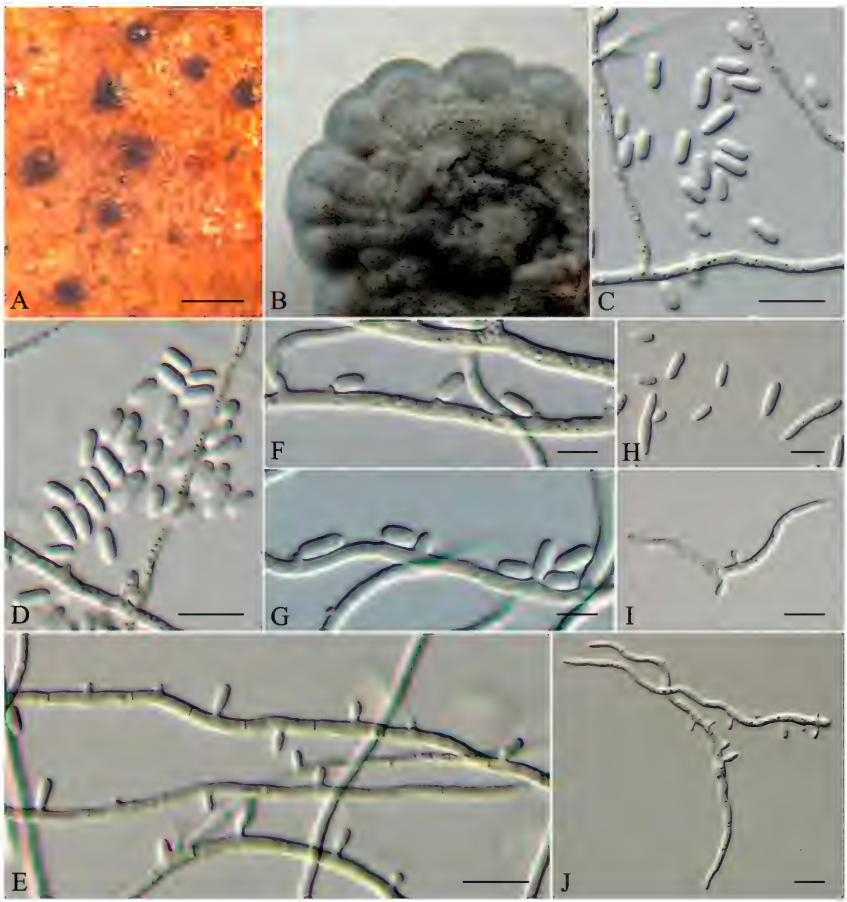

FIG. 2. *Peltaster fructicola* (LNHT1506). A. Signs on crabapple. B. Colony on PDA. C, D. Conidia and conidiogenous cells. E–G. Conidiogenous cells giving rise to conidia, with visible conidiogenous loci. H–J. Conidia expanding and germinating and microcyclic conidiation. Scale bars: A = 0.2 mm; C–E, H–J = 10 μm; F, G = 5 μm.

New Zealand) and Professor Zhongyi Zhang (College of Plant Protection, Yunnan Agricultural University, Kunming, Yunnan, China) for reviewing the manuscript.

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A new greenish gilled species of *Marasmius* (*Agaricales*) from Hainan Island, China

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Abstract —*Marasmius subviridiphyllus* is described as a new species from China supported by macro- and microscopic characters. It is classified in sect. *Marasmius* and characterized by its collariate greenish lamellae, developed rhizomorphs, slender fruiting bodies, dull red sulcate pileus with a small greenish white disc, middle sized basidiospores (8.8–10.6 × 3.9–5 μm), *Siccus*-type cheilocystidia, and pileipellis broom cells. The holotype (GDGM 26402) is deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM).

Key words — *Basidiomycetes, Marasmiaceae,* morphology, taxonomy

Introduction

Marasmius (*Basidiomycota, Marasmiaceae*) is a large genus with complex and diverse morphological characters, for which six sections are accepted (Wilson & Desjardin 2005). Representatives of sect. *Marasmius* are characterized by their collariate lamellae, insititious stipe, and hymeniform pileipellis with *Rotalis*- or *Siccus*-type broom cells. More than 200 species belong to this section (Singer 1964, 1976; Desjardin & Horak 1997, Desjardin & Ovrebo 2006, Antonín 2003, 2007; Antonín & Buyck 2006, Antonín & Noordeloos 2010).

Twenty-two species of *Marasmius* sect. *Marasmius* have been recorded in China (Bi et al. 1985, 1993, 1997; Chang & Mao 1995, Keissler & Lohwag 1937, Li & Bau 2003, Li et al. 1994, Mao 1998, Shao & Xiang 1997, Tai 1979, Teng 1936, Zhuang 2005). During a survey in Hainan Province, we discovered a new species belonging to sect. *Marasmius,* which we describe and illustrate here.

Materials & methods

Specimens were annotated and photographed in the field, dried in an electric drier at a temperature of 70°C, and then preserved in the herbarium. Spacing of lamellae spacing is accompanied by the number of lamellae reaching the pileus margin from the stipe, and the series number indicates the number of lamellulae between lamellae. Color terms and notations follow those of Kornerup & Wanscher (1978). Fungal tissues were mounted in 5% KOH and Melzer's reagent for microscopic examination. Spore statistics include: x_m, the arithmetic mean of the spore length by spore width (± standard deviation) for n spores measured in a single specimen; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, the number of spores measured per specimen; s, the number of specimens involved. Specimens are deposited in Fungal Herbarium of Guangdong Institute of Microbiology (GDGM). Authors of fungal names are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do).

Taxonomy

Marasmius subviridiphyllus Chun Y. Deng, Yi H. Yang & T.H. Li, **sp. nov.** Figs 1, 2

MycoBank MB800428

Differs from *Marasmius nigrobrunneus* by its dull red pileus, pale green lamellae, and narrower basidiospores.

Type: China, Hainan Province, Ledong County, Jiaxi Provincial Nature Reserve, 22 July 2009, Chun Y. Deng (**Holotype**, GDGM 26402).

Etymology: *sub-* = almost, *viridis* = green, *phyllum* = lamella; referring to the greenish lamellae.

Pileus 3–7 mm, hemispherical to convex when young, umbilicate to depressed at the center, deeply sulcate, glabrous, mostly reddish gray to dull red (8B2–3; 9B3), with a minute papilla at a small greenish white to greenish (29A2–3) disc. Lamellae collariate near to the stipe, or subfree with the broken collar when mature, distant (13–14), with 0–1 lamellulae, greenish white (29A2, 30A2) to pale green (29A3). Stipe 30–60 mm long, 0.2–0.5 mm thick, deep chestnut, brownish gray (8F2–3) to fuliginous or black, shiny, glabrous, insititious, equal, arising from black rhizomorphs.

Basidiospores 8.8–10.6 × 3.9–5 µm, [x_m = 9.3 × 4.8 µm, Q = 1.68–2.4, Q_m = 1.9, n = 20 spores, s = 1 specimen], ellipsoid, smooth, thin-walled, hyaline, inamyloid. Basidia 26–32 × 6–8 µm, clavate, 2- or 4- spored, thin-walled, hyaline, inamyloid. Basidioles 15–23 × 6–8 µm, fusoid, clavate, thin-walled, hyaline, inamyloid. Lamellae edge sterile. Cheilocystidia 10–30 × 6–11 µm, clavate, subcylindrical or irregular in outline, broom cells of the *Siccus*-type, with several apical cylindrical to conical appendages or branched setulae 1–7 × 1–1.5 µm, thin- or thick-walled. Pleurocystidia absent. Hyphae of hymenophoral trama and the pileus trama thin-walled, hyaline, dextrinoid,

Fig. 1: *Marasmius subviridiphyllus* (Holotype, GDGM 26402).
Scale bar = 1 cm. (Photo: Chun Y. Deng).

non-gelatinized. Stipitipellis consisting of parallel, thin-walled, brown, dextrinoid hyphae. Pileipellis a hymeniform layer of *Siccus*-type broom cells: main body 23–32 × 6–10 µm, cylindrical, subglobose to clavate, thin- to thick-walled, hyaline, inamyloid; apical setulae 2–7 × 1–2 µm, cylindrical to conical, rarely forked or warted, thin- to thick-walled, light brown in KOH, inamyloid. Caulocystidia absent. Clamp connections present in all tissues.

Ecology & distribution—Saprotrophic, gregarious on dead branches, sticks, etc. of dicotyledonous plants. In mixed forest, China (Hainan). July.

Comments— The main characters of *Marasmius subviridiphyllus* are the reddish gray to dull red, glabrous pileus, slender and shiny stipe, collariate pale green lamellae, black rhizomorphs, and *Siccus*-type pileipellis broom cells, and cheilocystidia. It belongs in sect. *Marasmius* subsect. *Sicciformes* according to Antonín (1991) and Wilson & Desjardin (2005). The pale green lamellae are distinctive within subsect. *Sicciformes*, because almost all known species in the subsection have white, yellowish white, or cream lamellae.

Among the species with tiny dark basidiomata, the new species resembles *M. nigrobrunneus* (Pat.) Sacc. and its provisional form *M. nigrobrunneus* "f. *cinnamomeus*," *M. ruforotula* Singer, and *M. subruforotula* Singer. *Marasmius*

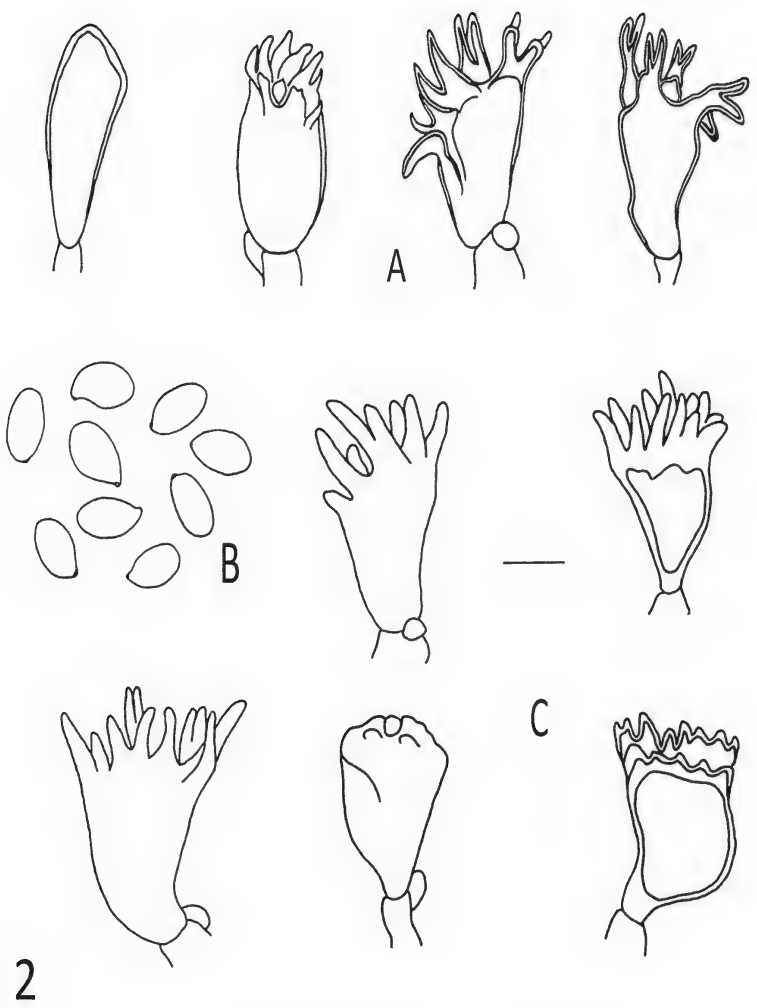

Fig. 2: *Marasmius subviridiphyllus* (Holotype, GDGM 26402).
A. Cheilocystidia; B. Basidiospores; C. Pileipellis. Scale bars = 10 μm.

nigrobrunneus is separated by a grayish brown pileus and broader basidiospores (5–6 μm) (Patouillard 1893, Singer 1958, 1976; Pegler 1983, 1986; Antonín 2007), and *M. nigrobrunneus* "f. *cinnamomeus*" differs in its brown to orangish brown pileus and the presence of smooth cells in the pileipellis and fewer broom cells (Wannathes et al. 2009); *M. subruforotula* differs in having smaller

broom cells in the pileipellis and cheilocystidia and coralloid cells among apical setulae (Singer 1964, Antonín & Buyck 2006). *Marasmius ruforotula* differs in closer (≤17) and marginate lamellae, longer stipe, absence of rhizomorphs, and inamyloid pileus and lamellar trama (Singer 1948, Wannathes et al. 2007)

Marasmius bekolacongoli Beeli, *M. grandiviridis* Wannathes et al., and *M. galbinus* T.H. Li & Chun Y. Deng resemble the new species in having green lamellae, but all three have a hymeniform pileipellis composed of smooth cells and belong to *Marasmius* sect. *Globulares* (Beeli 1928, Antonín 2007, Wannathes et al. 2009, Deng & Li 2011).

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MYCOTAXON

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A new species and new record of *Chloridium* from the Qinghai-Tibet Plateau Area, China

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Abstract — A new species, *Chloridium xigazense,* and a new record for China, *C. phaeosporum,* are described and illustrated. Specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP). Duplicates are kept in the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

Key words — dematiaceous hyphomycetes, soil fungi, taxonomy

Introduction

During the course of a survey of soil dematiaceous hyphomycetes in China, two *Chloridium* species were isolated. One represents a new species and the other is a new record for China. They are both described and illustrated from cultures grown on corn meal agar (CMA; Matsushima 1975).

The genus *Chloridium* was established by Link (1809) and is characterized by proliferating macronematous conidiophores. The conidiogenous cells are monophialidic, integrated, terminal, usually percurrent, and more or less cylindrical. Conidia, which are formed in slimy masses, are simple, ellipsoidal or subspherical, 0-septate, hyaline, and smooth. Index Fungorum (2012) lists 69 taxa; however, many of these are infraspecific, and Seifert et al. (2011) estimate that the genus may contain only 20 valid species.

Chloridium xigazense Y.M. Wu & T.Y. Zhang, **sp. nov.** Fig. 1

MycoBank MB 801786

Differs from *Chloridium smithiae* by its larger conidia and from *C. phaeosporum* by its frequently proliferating conidiogenous cells and hyaline conidia.

Type: China, Tibet, Xigaze, from a grassland soil, altitude 3600 m, 7 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$0874; **isotype** HMAS 196268).

Etymology: in reference to the type locality.

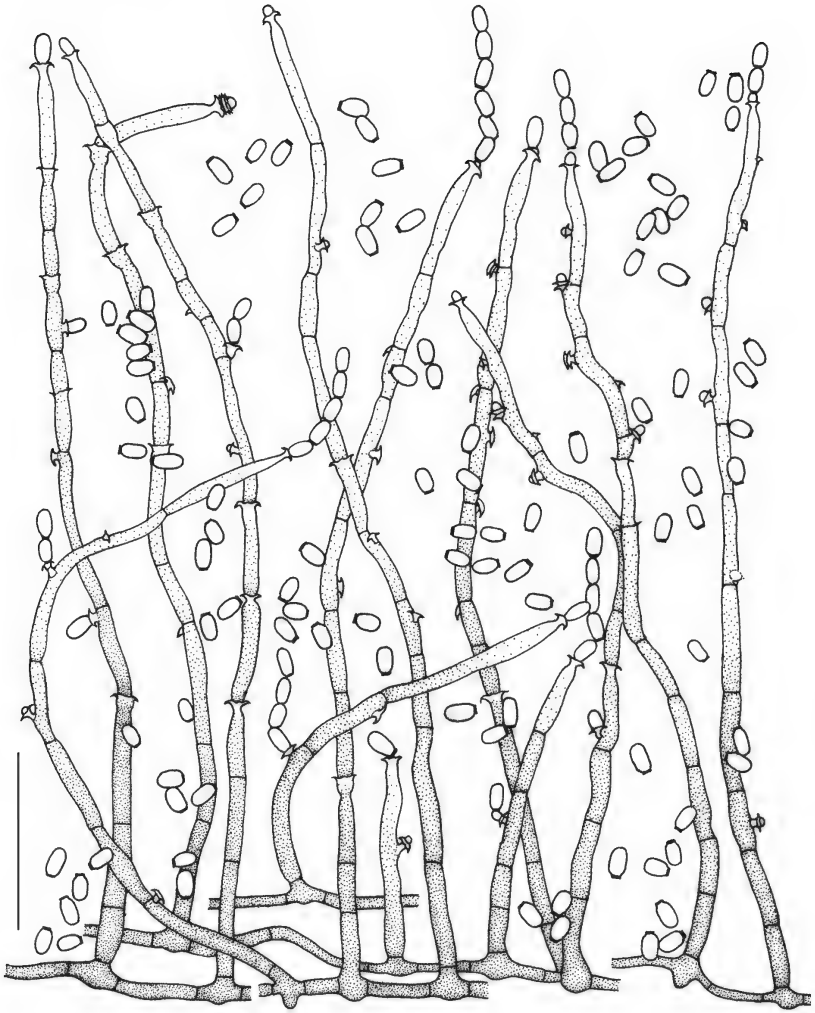

Fig. 1. *Chloridium xigazense* (ex holotype).
Conidia, conidiophores and conidiogenous cells. Scale bar = 25 μm.

Colonies on CMA broadly effuse, brown to dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, subhyaline to light brown, 2–3 μm wide hyphae. Conidiophores brown, paler towards the apex, macronematous, mononematous, solitary or in groups, erect, septate, smooth, 80–140 μm long, 2–3 μm wide. Conidiogenous cells monophialidic, terminal, constricting abruptly and expanding in a flaring collarette, thereafter frequently proliferating 1–4 times. Conidia produced singly but often adhering

in chains, 0-septate, smooth, hyaline, ellipsoidal to oblong, obtuse at the apex, with a dark hilum at the subtruncate base, 3–5 × 2–2.5 µm.

Comments – Morphologically, *C. xigazense* resembles *C. smithiae* R.C. Sinclair & Eicker (Sinclair & Eicker 1985) and *C. phaeosporum* (Gams & Holubová-Jechová 1976), but *C. smithiae* has smaller conidia (2–3 × 1–1.7 µm) while *C. phaeosporum* shows infrequent proliferation of the conidiogenous cells and its conidia are pigmented.

Chloridium phaeosporum W. Gams & Hol.-Jech., Stud. Mycol. 13: 27, 1976 Fig. 2

Colonies on CMA effuse, olivaceous brown, velvety. Mycelium superficial or immersed; hyphae branched, septate, smooth, pale to mid brown, 1.5–2.5 µm wide. Conidiophores mid brown, paler towards the apex, macronematous,

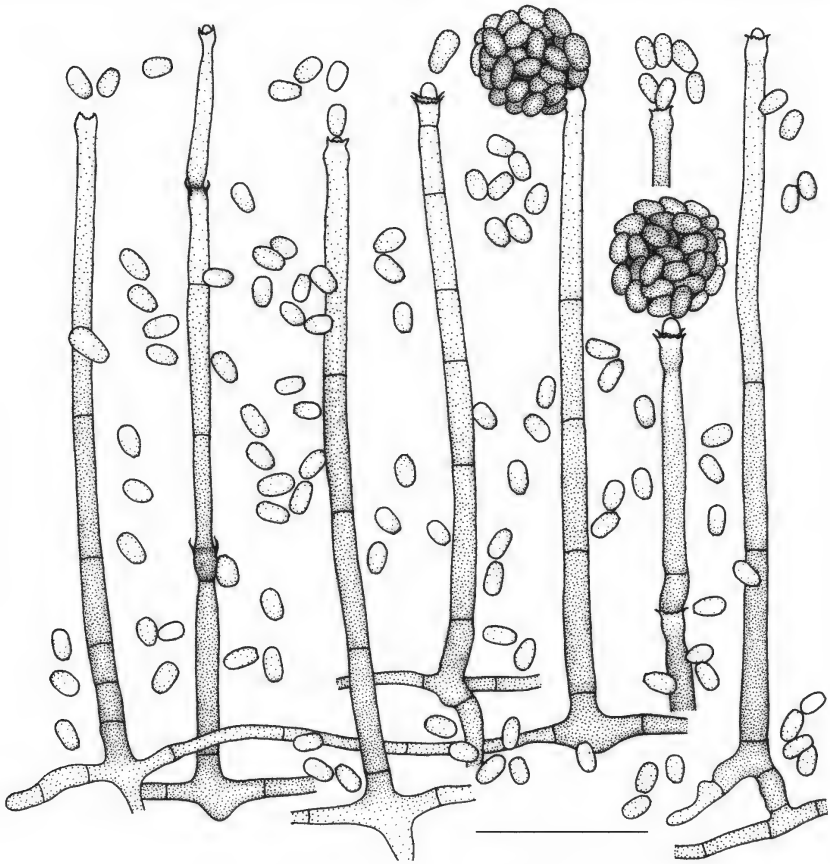

Fig. 2. *Chloridium phaeosporum* (HSAUP II $_{07}$1037).
Conidia, conidiophores and conidiogenous cells. Scale bar = 25 µm.

mononematous, solitary or in groups, erect, septate, smooth, 70–120 μm long, 2.5–3.5 μm wide. Conidiogenous cells monophialidic, terminal, constricting abruptly and expanding in a flaring collarette, rarely proliferating. Conidia aggregated in heads, 0-septate, smooth, pale brown, ellipsoidal to oblong, obtuse at the apex, with a dark hilum at the subtruncate base, 3–6 × 1.5–2.5 μm.

Specimen examined: **CHINA**, **Tibet,** Xigaze, altitude 3000 m, from a mountain soil, 10 Sept. 2007, Y.M. Wu (HSAUP II $_{07}$1037, HMAS 196269).

Chloridium phaeosporum is reported for the first time from China. Compared with the morphological characters of the species as described by Gams & Holubová-Jechová (1976), both collections have pale brown conidia that are ellipsoidal to oblong, 0-septate, obtuse at the apex, with a dark hilum at the subtruncate base, and measure 3–6 × 1.5–2.5 μm. We believe they are the same species. *Chloridium phaeosporum* is most similar to *C. virescens* (Pers.) W. Gams & Hol.-Jech. var. *virescens* (Gams & Holubová-Jechová 1976) in conidial shape, but the latter species has hyaline conidia without a dark basal hilum.

Acknowledgments

The authors are grateful for pre-submission comments and suggestions provided by Dr. Eric McKenzie, Prof. Y.L. Guo, and Dr. Shaun Pennycook. This project was supported by the National Science Foundation of China (no. 30970011 & 30499340).

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A new species of *Heliocephala* from Vietnam

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Abstract —*Heliocephala vietnamensis* sp. nov., a hyphomycetous fungus collected on decaying leaves of an unidentified plant, is described and illustrated. It is distinguished by determinate apical cluster of conidiogenous cells and obclavate to sub-navicular, 3-septate, pale brown conidia.

Key words — anamorphic fungi, systematics, leaf litter

Several samples of dead plant material colonized by anamorphic fungi were collected in a dense monsoon tropical forest in Vietnam. Among these samples was a conspicuous *Heliocephala* species, which is herein described and illustrated.

Taxonomy

Heliocephala vietnamensis Melnik & R.F. Castañeda, **sp. nov.** Figs 1,2

MycoBankMB801539

Differs from *Heliocephala elegans* and *H. triseptata* by conidial shape, number of septa and size.

Type: Vietnam, Dong Nai, Cat Tien National Park, right bank of the Dong Nai River, near to *Ficus* sp. plot, a dense monsoon tropical forest, 11°26′N 107°25′E, on decaying leaves of unidentified plant, 2 January 2011, coll. Yu. Novozhilov, (**Holotype**: LE 261853).

Etymology: Latin, *vietnamensis*, refers to the country where the fungus was found.

Colonies on the natural substrate effuse, hairy, amphigenous, brown. Mycelium mostly superficial composed of septate, branched, dark brown, smooth-walled

Fig 1. *Heliocephala vietnamensis* (ex holotype LE 261853). A. Conidia. B. Conidiophore, conidiogenous cell and attached conidia. Scale bars = 10 μm.

hyphae, 1.8–2.8 μm diam. Conidiophores distinct, single, cylindrical, erect, straight, 210–340 × 6–8 μm, 14–16 μm wide at the base, 7–12-septate, bearing a conidiogenous apparatus at the apex consisting of 3–6 irregular-campanulate

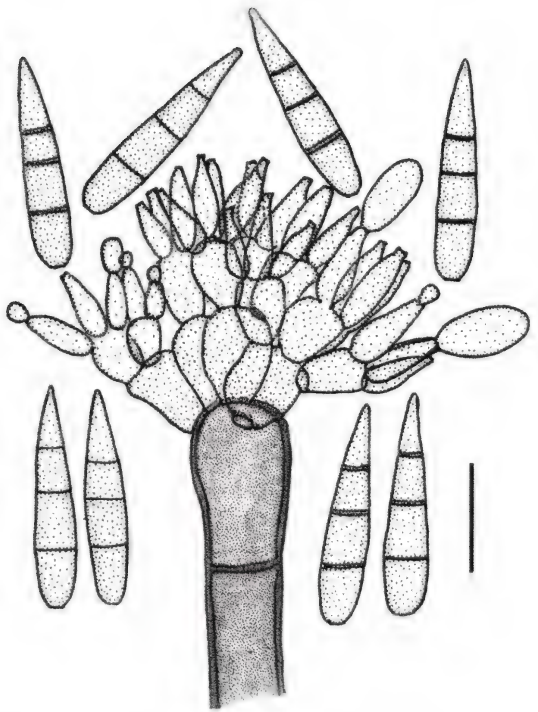

Fig 2. *Heliocephala vietnamensis* (ex holotype LE 261853). Conidiogenous cells and conidia. Scale bar = 10 μm.

to irregular-doliiform or cuneiform primary and secondary branches (metula-like), pale brown, 3–4 × 3.5–4.0 μm. Conidiogenous cells monoblastic, discrete, determinate, delicate, lageniform, 1.7–3.0 × 1.5–2.5 μm, pale brown, smooth, forming a compact cluster at the ends of the branches. Conidial secession schizolytic. Conidia solitary, acrogenous, obclavate to navicular, 3-septate, pale brown, 14–17 × 2.8–3.8 μm, smooth-walled, dry. Teleomorph unknown.

Note: The genus *Heliocephala* V. Rao et al. (Rao et al. 1984) was based on *H. proliferans*, which was distinguished by the production of compact clusters of discrete, lageniform, monoblastic conidiogenous cells arising more less radially or closely fasciculate from short secondary or tertiary branches (metula-like) at the apex of the conidiophores. The conidia are obclavate, long or short rostrate, septate, pale brown, with an unusual germination of the apical cell to form a secondary cluster of conidiogenous cells.

Heredia et al. (2011), who emended *Heliocephala* after molecular and morphological studies of some *Heliocephala* species and the closely similar *Holubovaniella* R.F. Castañeda (Castañeda 1985), considered *Holubovaniella* a synonym of *Heliocephala*. They provided a key to the species and expanded the generic concept of *Heliocephala* to accommodate taxa with indeterminate conidiophores with several extensions of the main axis and solitary conidia produced by the same kind of conidiogenesis. Following Heredia et al. (2011), *Heliocephala* comprised six species: *H. elegans* (R.F. Castañeda) R.F. Castañeda & Unter., *H. gracilis* (R.F. Castañeda) R.F. Castañeda & Unter., *H. natarajanii* Kumaresan & M. Sriniv., *H. proliferans* V. Rao et al., *H. triseptata* Heredia et al., and *H. zimbabweensis* Decock et al.

Among the previously described *Heliocephala* species, only *H. elegans* and *H. triseptata* are similar to *H. vietnamensis*, but *H. elegans* (Castañeda-Ruiz 1985) has indeterminate conidiophores with several clusters of short branches and intercalary conidiogenous cells on the axis before each extension and olivaceous conidia that are obclavate to lecythiform with a shortly rostrate apical cell, mostly 2-septate, 8–25 × 3–4 µm. *H. triseptata* (Heredia et al. 2011) has conidia that are obclavate or navicular to broadly fusiform, rostrate, 15–27 × 3.5–4.5 µm with a 10–14 µm long rostrum. Neither species can be confused with *H. vietnamensis*.

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The authors express their sincere gratitude to Dr. Bryce Kendrick and Dr. De-Wei Li for their critical review of the manuscript. We are deeply indebted to Dr. Yuri Novozhilov for providing the specimen collected. In part this study has been done in frame of Research Program of the Russian-Vietnamese Tropical Research and Technological Center is also appreciated. We also thank Dr. Gabriela Heredia for facilities during microscopic observations and photographs. The authors are deeply indebted to the Russian Academy of Sciences and the Cuban Ministry of Agriculture for facilities. We also thank Mirtha Caraballo and Beatriz Ramos for technical assistance. We acknowledge the facilities provided by Dr. P.M. Kirk and Drs. V. Robert, G. Stegehuis and A. Decock through the maintenance of the Index Fungorum and Mycobank websites. Dr. Lorelei L. Norvell's editorial review and Dr. Shaun Pennycook's nomenclatural review are greatly appreciated.

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Lecanora gansuensis sp. nov. (*subfusca* group) from China

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Abstract –– *Lecanora gansuensis* is described as new to science from China and South Korea. It is characterized by flexuous apothecial margins and the presence of stictic acid, norstictic acid, and connorstictic acid in addition to atranorin.

Key words –– East Asia, fungi, lichen, stictic acid complex, taxonomy

Introduction

The large genus *Lecanora* Ach. has been divided into several groups. The *subfusca* group is characterized by having atranorin, calcium oxalate crystals in the apothecial margin, and (usually) reddish brown apothecia (Brodo 1984). The *subfusca* group has received considerable interest during recent decades (e.g., Brodo 1984, Miyawaki 1988, Brodo et al. 1994, Upreti 1998, Guderley 1999, Lumbsch et al. 2004, Ryan et al. 2004). A further study of *Lecanora* specimens collected from China revealed a new species belonging to the *subfusca* group, which is described here.

Materials & methods

The specimens studied are housed in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University) or KoLRI (Korean Lichen Research Institute). Thalli were examined and measured under a stereo–microscope (COIC XTL7045B2) and apothecial anatomy was observed under a polarizing microscope (Olympus CX41–32). Photos of the thalli were taken with an Olympus SZX12 with DP70. Chemical analysis using spot tests and thin layer chromatography followed standard methods (Orange et al. 2010), mainly using solvent system C.

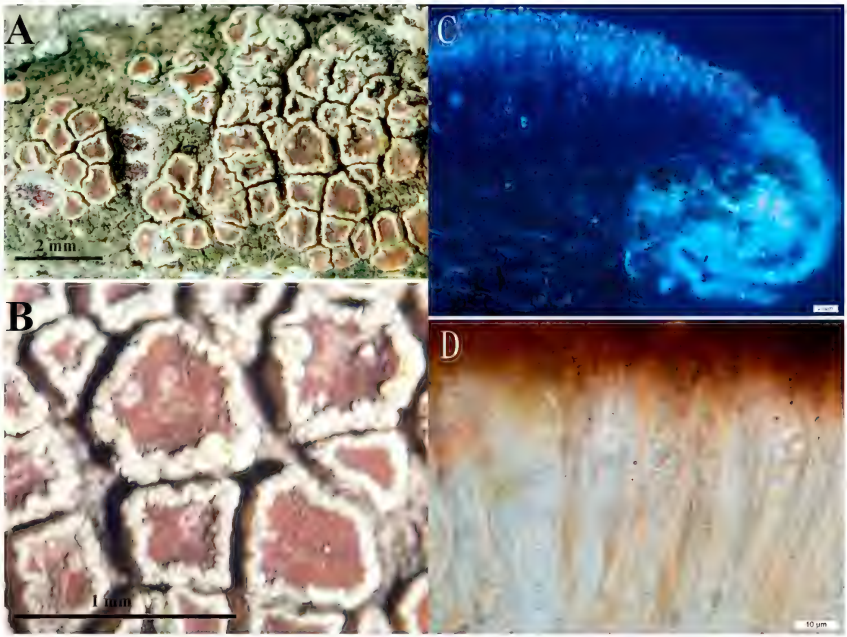

Plate 1. *Lecanora gansuensis* (holotype). A. Crowded apothecia; B. Flexuous apothecial margins; C. Granules in amphithecium and epihymenium; D. Ascospores.

Taxonomy

Lecanora gansuensis L. Lü & H.Y. Wang, **sp. nov.** Plate 1

MycoBank 563751

Differs from *Lecanora kansriae* by its crowded flexuous apothecia and smaller spores.

Type: China, Gansu, Wen County, Qiujiaba, 2300 m, on twigs, 3 Aug 2006, Yang et al. 061482–2 (SDNU **holotype**).

Etymology: the epithet refers to the holotype locality, Gansu.

Thallus dark brown, well–developed, continuous or rimose–areolate, margin definite, prothallus absent. Apothecia sessile, crowded, 0.5–1.0 mm in diam., discs red brown, epruinose, margins gray–white, prominent, entire, thick, strongly flexuous. Cortex is distinct, thin, gelatinous, uniform below (15–23 μm). Amphithecium containing small crystals (*campestris*–type) insoluble in KOH. Epihymenium yellowish brown, with fine granules (*pulicaris*–type), 12.5–15 μm tall. Hymenium hyaline, 50–60 μm tall, paraphyses simple, to be slightly branched at the tip, neither thickened apically nor pigmented. Hypothecium hyaline, 45–55 μm thick. Asci clavate, 8–spored. Ascospores simple, hyaline, ellipsoid, 8.5–10 × 5–5.5 μm.

Chemistry — atranorin (major), stictic acid (major), norstictic acid (minor), and connorstictic acid (minor).

Additional specimens examined: **CHINA. Gansu**: Wen County, Qiujiaba, 2350 m, on bark, 3 Aug. 2006, Yang et al., 061643; 2600 m, Yang et al., 062075–2. **Guizhou**: Kaili City, Mt. Leigong, 1700m, on bark, 23 Aug. 2010, Wang 20103351; Libo County, Maolan Nature Reserve, 350m, on bark, 2 Nov. 2009, Wang 20102329 (SDNU). **SOUTH KOREA. Jeollanam-do**: Gurye-gun, Sandong-myeon, Mt. Jiri, 1085 m, on bark, 16 Jun. 2006, Hur 060165–3 (KoLRI).

Distribution and substrate — At present, *L. gansuensis* is known from China (Gansu and Guizhou) and South Korea on bark or wood.

Comments — Few species of *Lecanora* have a *pulicaris*-type epihymenium with a *campestris*-type amphithecium, and even fewer contain stictic acid as a main lichen product. *Lecanora kansriae* described from Thailand agrees in all these respects but has much larger spores (17–20 × 10–12 μm) and does not have crowded apothecia with flexuous margins (Papong & Lumbsch 2011). *Lecanora gansuensis* morphologically resembles *L. allophana* (Ach.) Ach. and *L. imshaugii* Brodo. However, *L. allophana* has a *glabrata*-type epihymenium and different substances (atranorin alone), while *L. imshaugii* differs in chemistry, producing atranorin and zeorin as major substances (Brodo 1984).

Acknowledgements

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A new species of *Gautieria* from China

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Abstract —*Gautieria xinjiangensis,* collected from Xinjiang, China, is described and illustrated as new to science.

Key words —*Gomphaceae*, new taxon, morphology, taxonomy

Introduction

The diagnostic characteristics of *Gautieria* Vittad. (*Gomphaceae*, *Basidiomycota*) are a subglobose to reniform fruit body with a thin and easily evanescent peridium, a cartilaginous gleba with simple or branched columella and a coralloid type of development, often elongated, labyrinthiform cavities, a gelatinous-cartilaginous tramal plate and basidiospores with longitudinal ribs (Cunningham 1944, Zeller 1948, Rauschert 1975, Beaton et al. 1985). About 36 species and varieties have been described, with nine species known from China (Liu 1998, Ying 1984, 1995).

Recently, we have encountered an additional species, which is proposed here based on morphological characters.

Materials & methods

Materials were collected at Tianshan Mountains of Xinjiang, northwestern China and were deposited in the Herbarium of Mycology of Jilin Agricultural University (HMJAU). Morphological characters of the fungus were described and illustrated with traditional taxonomic methods. 5% KOH solution and Melzer's reagent were used when examining the microscopic characters.

Taxonomy

Gautieria xinjiangensis T. Bau, **sp. nov.** Plates 1–2

MycoBank MB 801448

PLATE 1. Basidiomata of *Gautieria xinjiangensis* (holotype).

Differs from *Gautieria morchelliformis* by its larger basidiomata and its broadly ellipsoid to orbicular ovate basidiospores.

TYPE: China, Xinjiang Autonomous Prefecture, Urumchi City, Tianshan Mountains, on ground in forest dominated by *Picea schrenkiana* Fisch. & C.A. Mey., 5 September 2005, Tolgor Bau (**Holotype,** HMJAU6009; GenBank, JX860192).

ETYMOLOGY: *xinjiangensis* refers to the type locality.

MACROCHARACTERS — Basidioma emergent, 3–5.5 × 3–4 cm, subglobose to ovoid in outline, surface brain-like, yellow-brown to light rust; basal rhizomorph whitish, distinct. Peridium practically absent, or barely visible. Gleba yellow-brown to yellow-tan; columella absent; locules irregularly shaped, labyrinthine; inner surface covered with yellow-brown or yellow-tan hymenium.

MICROCHARACTERS — Basidiospores (13–)15–19(–21) × (8–)8.5–10(–11) µm, Q = 1.8–2, broadly ellipsoid to orbicular-ovate, yellow-brown to pale brown in KOH, with 9–11 longitudinal ribs, sometimes with branches or cross veins between ribs; apex broadly round; apiculus long, 2–3 × 1.5–2 µm. Basidia 20–30 × 7–10 µm, clavate, with granular or oil drops, 4- or 2-spored; sterigmata 2–3 µm long. Cystidia 30–40 × 8–12 µm, clavate, thin-walled to slightly thick-walled, hyaline, colorless. Peridium traces can be seen under a microscope, and composed of pseudo-parenchyma of large, subglobose to polygonal cells, usually 30–40 µm in diam, thin-walled, smooth, hyaline, frequently septate (cells 12–25 µm long). Tramal hyphae gelatinized, interwoven, frequently septate and branching, 3–7 µm diam., smooth, hyaline. Clamps absent in all tissues.

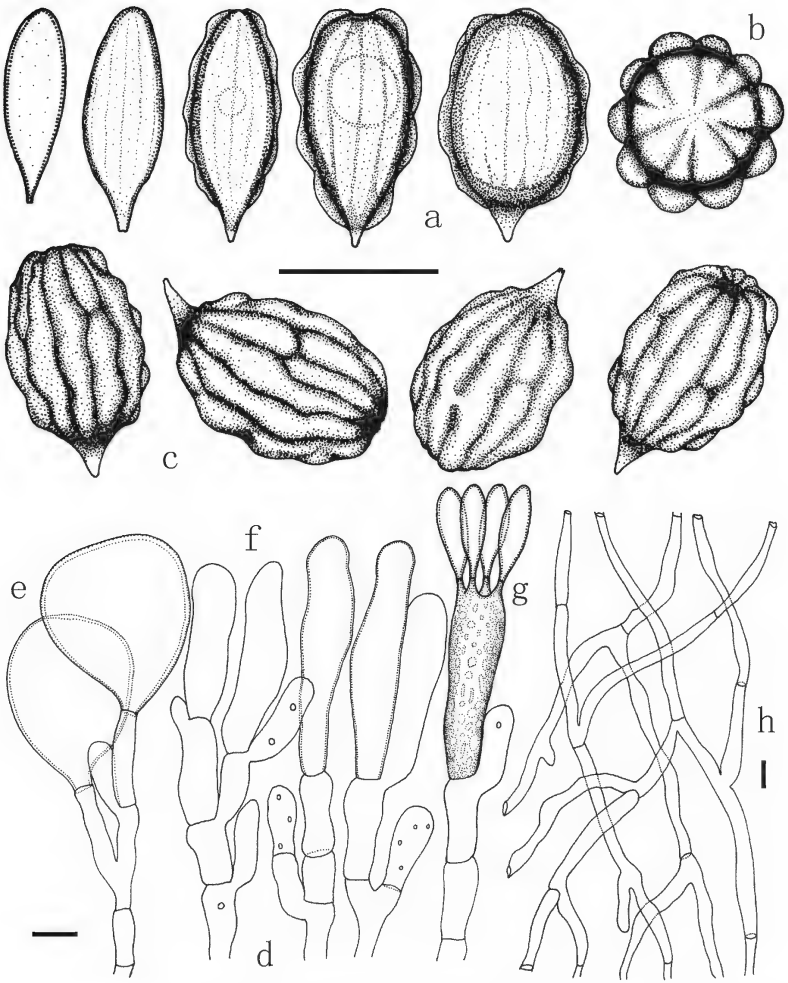

Plate 2. Microscopical characters of *Gautieria xinjiangensis* (holotype). a: young basidiospores; b: mature basidiospore in polar view; c: mature basidiospores; d: hymenium; e: pseudo-parenchyma cells of peridium traces; f: cystidia; g: basidium with young basidiospores; h: tramal hyphae. Scale bar = 10 μm.

Discussion

The main characteristics of *G. xinjiangensis* are the relatively large basidioma with a white basal rhizomorph, the absence of a columella, and the broadly ellipsoid basidiospores with 9–11 longitudinal ribs.

The nine *Gautieria* species reported from China (Liu 1998, Ying 1984, 1995) are all very different from the new taxon according to the authors' review of

the literature and examination of voucher specimens. *Gautieria hubeiensis* K. Tao et al. and *G. morchelliformis* Vittad. are distinguished by their lemon-shaped to long-ellipsoid basidiospores, and *G. globispora* K. Tao et al. by its globose basidiospores (Liu et al. 1996, Pegler et al. 1993, Tao et al. 1996, Vittadini 1831, Zeller & Dodge 1918). The other six species (*G. pallida* Harkn., *G. shennongjiaensis* K. Tao et al., *G. macrospora* G. Cunn., *G. sinensis* J.Z. Ying, *G. chengdensis* J.Z. Ying, *G. gautierioides* (Lloyd) Zeller & C.W. Dodge) differ from *G. xinjiangensis* by their smaller basidiomata (Liu 1998). In addition, *G. xinjiangensis* is associated with *Picea schrenkiana*, a plant endemic to Tianshan Mountains in China.

The ITS sequence of the holotype of *G. xinjiangensis* (Genbank JX860192) is highly (<99%) similar to sequences of USA material of *G. morchelliformis* (GenBank AF377066, AF377067), but the two species clearly differ in their basidiospore shape and basidioma size.

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Typification of *Tuber formosanum* (*Tuberaceae, Pezizales, Ascomycota*) from Taiwan, China

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Abstract—When Hu described *Tuber formosanum* in 1992 as a new species based on collections from Dung-Pu, Nantou County, Taiwan, he neglected to designate a type, so that the name was not validly published. The current authors re-describe, illustrate, and validate *T. formosanum* based on a type selected from recent collections from the same region. *Tuber formosanum* is closely related to the *indicum* complex but differs in morphological, phylogenetic, and other respects and should be treated as a separate taxon.

Key Words—truffle, taxonomy, ITS

Introduction

A few ascocarps of *Tuber* species were collected from the natural forest of *Cyclobalanopsis glauca* (Thunb.) Oerst. (*Fagaceae*) in Dung-Pu, Nantou County, Taiwan, in late October 1987, mid-December 1988, early February 1990, and early January 1991. They grew in calcareous soils (pH 7.0) under *Cyclobalanopsis glauca* trees at 1300 m elevation. Hu (1992) described *Tuber formosanum* as a new species based on these collections. Because Hu did not designate a type in his description, the new species was not validly published (McNeill et al. 2006: Art. 37.1). Recent phylogenetic and morphological analyses of *T. formosanum* collections confirm that *T. formosanum* is an independent species, although closely related to the *T. indicum* complex. In addition, it has different host plants and is restricted to Taiwan. Consequently, *T. formosanum* should be treated as a separate taxon (Huang et al. 2009). In this paper, we re-describe,

Plate 1. Map of Taiwan showing where the type of *Tuber formosanum* was collected [adapted from Huang et al. 2009].

illustrate, and validate *T. formosanum* based on a type newly selected from specimens collected from the Ho-She district of National Taiwan University Experimental *C. glauca* Plantation, Nantou County, Taiwan. The plantation was established with trees inoculated by *T. formosanum* from the natural *C. glauca* forests in Dung-Pu, Nantou County. The distance between Ho-She plantation (23°35′01″N 120°51′36″E) and the original natural *C. glauca* forest in Dung-Pu (23°30′27″N 120°53′45″E) is 9.21 km (Pl.1).

Table 1: The ITS sequences of *Tuber* species used in this study.

Taxon	Voucher or code	Geographical origin	GenBank
T. melanosporum	A71	Spain	AF106877
	Tm13	Vaucluse, France	AF132501
	MelW	Unknown	AF300825
T. sinense	Tsin-hd01	Huidong, Sichuan, China	DQ375526
	Tsin-hd02	Huidong, Sichuan, China	DQ375527
	KUN-HKAS44319	Huidong, Sichuan, China	GU979061
	IFS89925	Huidong, Sichuan, China	GU979062
T. indicum	KUN-HKAS44999	Kunming, Yunnan, China	GU979066
	KUN-HKAS49746	Yimen, Yunnan, China	GU979067
	KUN-HKAS41312	Haikou, Yunnan, China	GU979069
	KUN-HKAS30261	Panzhihua, Sichuan, China	GU979050
	KUN-HKAS30262	Panzhihua, Sichuan, China	GU979051
T. formosanum	KUN-HKAS48268	Ho-She, Nantou, Taiwan	GU979048
	KUN-HKAS49707	Ho-She, Nantou, Taiwan	GU979049
	KUN-HKAS62628 (holotype)	Ho-She, Nantou, Taiwan	JN655530
T. aestivum	E5	Molise, Italy	AF516791

Materials & methods

The macroscopic and microscopic characters were described based on the type selected from new specimens collected from Nantou County, Taiwan in 2005. The methods followed those of Yang & Zhang (2003). Sections were made with a razor blade, mounted in a 5% KOH solution and examined under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto doubled-sided tape, mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a JEOL, JMS-5600LV SEM. The new specimens were deposited at the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

DNA was extracted from ascomata using a modified CTAB protocol (Doyle 1987). The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the rDNA ITS region. PCR reaction solution and cycling parameters used by Chen & Liu (2007) were adopted and modified when necessary. The PCR reaction was performed on a Takara TP100 thermal cycler. Amplification products were electrophoresed on a 1% agarose gel, and purified with Sangon's purification kit. Sequencing was performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL automatic sequencer. Sixteen partial internal transcribed spacer ribosomal DNA (ITS-rDNA) sequences of *Tuber* species, *T. formosanum, T. melanosporum*, *T. sinense*, *T. indicum* and *T. aestivum* were used for analysis. *Tuber aestivum* was selected as outgroup (Table 1). Software and methods used in sequence alignment and phylogenetic analysis followed Chen & Liu (2007).

Taxonomy

Tuber formosanum H.T. Hu & Y. Wang, **sp. nov.** PLATE 2

MYCOBANK MB 563693

"*Tuber formosanum*" H.T. Hu, Quart. J. Exp. Forest Nat. Taiwan Univ. 6: 79–86 (1992), nom. inval. [No holotype designated].

Differs from *T. indicum* by its asci with short stipitate and spiny-reticulate ascospores, and its association with *Cyclobalanopsis glauca*.

TYPE: Taiwan, China: Nantou County, Ho-She, (E120°51′, N23°35′), in a *Cyclobalanopsis glauca* plantation, alt. 1200 m, 2005, H.D. Hu (**Holotype**, KUN-HKAS 62628; GenBank , JN655530).

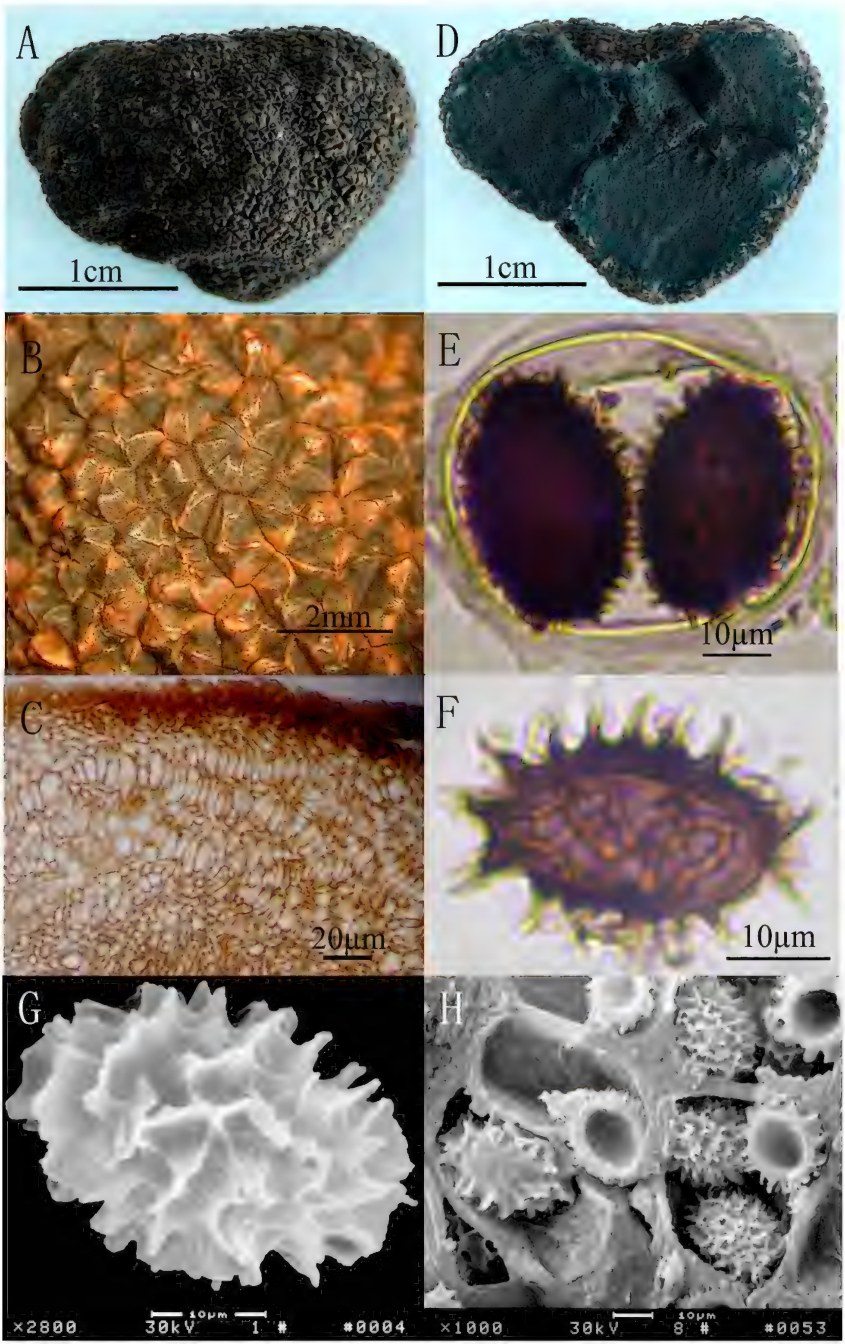
ETYMOLOGY: from the Latin *formosanum* referring to Taiwan, the location of the type collection.

ASCOMATA globose to subglobose and slightly lobed, firm, dark reddish brown to dark grayish brown, up to 8 cm in diameter, with low pyramidal warts, pentagonal, having 4–5 ridges, up to 1 mm high. ODOR slightly aromatic when mature. PERIDIUM 215–815 µm thick, variable, pseudoparenchymatous, composed of two layers: outer layer (50–)135–300(–465) µm thick, composed of angular or irregular cells (4–)8–39(–50) × (4–)5–15(–20) µm in diam., with thick dark brown walls of 0.5–2 µm; inner layer (50–)100–135(–560) µm thick, composed of hyaline to yellowish, narrow polygonal cells, merging with glebal tissue of interwoven hyphae. GLEBA solid, whitish, light yellowish when young, becoming brown to dark purplish brown at maturity, marbled with distinct, whitish, meandering veins merging at many points and sometimes penetrating through the peridium. ASCI globose or broadly elliptic, (37–)45–65(–85) × (26–)30–55(–69) µm (n = 160), with short stipitate, 1–4(–5) spored, randomly dispersed in glebal tissue. ASCOSPORES ellipsoid, whitish to yellowish when young, becoming dark brown at maturity, (27–)29–45(–48) × 20–32(–35) µm (1-spored), (26–)27–36(–39) × (18–)19–24(–28) µm (2-spored), 24–34 × (16–)18–23(–25) µm (3-spored), (25–)26–32(–33) × (17–)18–22 µm (4-spored) (excluding ornamentation, n = 40), Q = (1.17–)1.27–1.62(–1.70) (n = 170), somewhat spiny-reticulate, spines up to 2–5(–6) µm (n = 170) tall, wall (2–)3–5 (–6) µm (n = 160) thick.

ECOLOGY & DISTRIBUTION: Hypogeous in calcareous soils with pH 7.0 under *C. glauca* trees at 1200–1300 m a.s.l., fruiting from late October to early February. Known only from Nantou County, Taiwan, China.

ADDITIONAL SPECIMENS EXAMINED: **TAIWAN, CHINA. NANTOU COUNTY,** Ho-She, 23°35′N 120°51′E, in *Cyclobalanopsis glauca* plantation, alt. 1200 m, 2005, HD. Hu (KUN-HKAS48268, KUN-HKAS49707).

PLATE 2. *Tuber formosanum* (holotype, KUN-HKAS62628). A. Ascoma; B. Warts on peridium surface; C. Part of a section of peridium; D. Sectioned ascoma showing the gleba; E. Ascus with two ascospores inside; F. Ascospore showing the ornamentation; G, H. Scanning microscopic photos of ascospores (bars = 10µm).


A
1cm
D
1cm
B
2mm
E
10μm
C
20μm
F
10μm
G
×2800 30kV 1 #0004
H
×1000 30kV 8 #0053

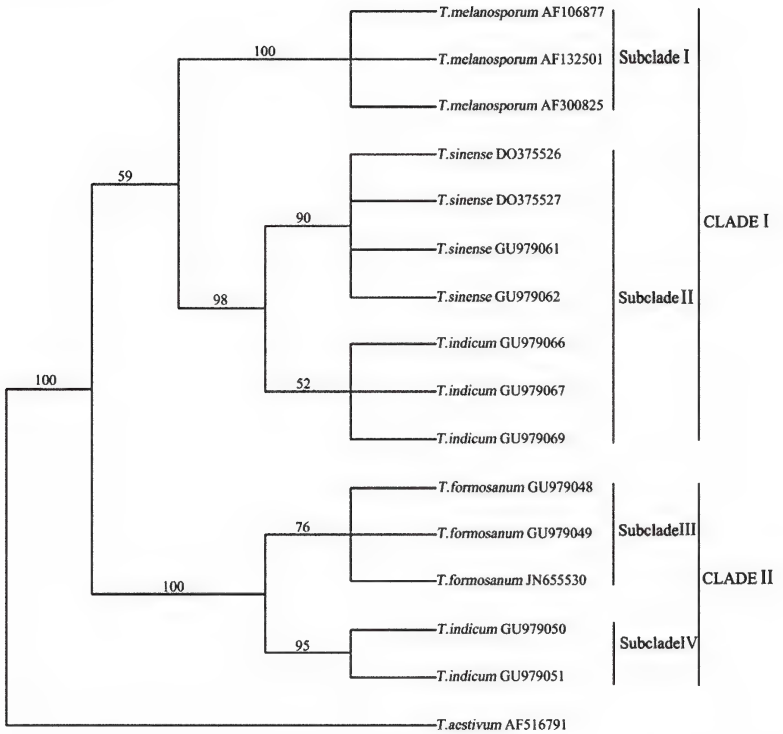


Plate 3: Strict consensus of equally parsimonious trees derived from the analysis of ITS1/5.8S/ITS2 sequence data (Length = 259steps; CI = 0.942; RI = 0.964). Numbers above branches indicate bootstrap support above 50%.

Phylogenetic analysis

The ITS phylogenetic tree revealed two major clades. Clade I divided into two subclades: subclade I corresponded to *T. melanosporum* and subclade II to *T. indicum* from Huidong, Sichuan and Yunnan prov. with high bootstrap support (≥98%). Clade II also divided into two subclades; subclade III corresponded to *T. formosanum* and subclade IV to two *T. indicum* isolates from Panzhihua, Sichuan prov. The three sequenced specimens of *T. formosanum* formed a subclade with bootstrap support of 76% (Pl.3).

Discussion

The Taiwanese truffle *Tuber formosanum* is closely related to other Asian black truffle species, such as *T. indicum* Cooke & Massee and *T. sinense* K. Tao & B. Liu (Chen 2007; Huang et al. 2009; Chen et al. 2011). However, recent phylogenetic and morphological analyses of these taxa show that although *T. formosanum* is closely related to *T. indicum* and *T. sinense*, it should still be

treated as a distinct species indigenous to Taiwan (Huang et al. 2009; Chen et al. 2011; Kinoshita et al. 2011). *Tuber formosanum* differs from *T. indicum* by its asci with short-stipitate spiny-reticulate ascospores. Most *T. indicum* asci lack stipes and its ascospore surfaces are more spike-like. *Tuber formosanum* associates naturally only with *Cyclobalanopsis glauca,* while *T. indicum* associates with both coniferous and broad-leaved trees, such as pine and chestnut. Because the original type specimen on which *T. formosanum* was based does not exist, we designate as type KUN–HKAS62628 from among specimens newly collected at the Ho-She district of National Taiwan University Experimental *C. glauca* Plantation, Nantou County, Taiwan. The plantation was established with the trees inoculated by *T. formosanum* gathered from the natural forests in Dung-Pu, Nantou County, Taiwan. The results of morphological and molecular analyses of samples from KUN–HKAS62628, KUN–HKAS48268 and KUN–HKAS49707 (Plate 3) were identical to those of Huang et al. (2009).

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Three new *Inocephalus* species with cuboid basidiospores from New South Wales and Queensland, Australia

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Abstract — Three new *Inocephalus* species with cuboid spores are described from central New South Wales and northern Queensland, Australia. *Inocephalus plicatus* is diagnosed by its yellow pileus becoming plicate-striate and yellowish orange when mature, pungent odor, bitter taste, and pseudocystidia on all lamellar surfaces. *Inocephalus hypipamee* is differentiated by its brown umbonate pileus, white lamellae and stipe, obclavate cheilocystidia, subisodiametric basidiospores and clampless hyphae. *Inocephalus parvisporus* possesses small basidiospores and a *Leptonia*-like stature.

Key words — *Basidiomycota*, *Entolomataceae*, LSU, mtSSU, RPB2

Introduction

The *Entolomataceae* Kotl. & Pouzar (*Agaricomycetes*, *Basidiomycota*) is a highly diverse euagaric family that includes more than 1500 species (Noordeloos & Gates 2012). Three genera are typically recognized: *Clitopilus* (Fr. ex Rabenh.) P. Kumm., *Rhodocybe* Maire, and *Entoloma* (Fr.) P. Kumm. sensu lato. Species of *Entolomataceae* are difficult to diagnose to genus and the proposed concepts of classification typically recognize either 13 subgenera within a single genus *Entoloma* sensu lato (Noordeloos 1992, 2004) or 13 genera including *Entoloma* sensu stricto (Largent 1994). Several phylogenetic studies were recently undertaken to produce a framework for placing species into genera or subgenera (Co-David et al. 2009, Baroni et al. 2011, Baroni & Matheny 2011). Although these studies have provided an important context for

framing ongoing debates of evolutionary relationships among species, most of the monophyletic subgroupings lack statistical support.

Without a stable system of classification, newly described species of *Entolomataceae* are classified into genera or subgenera using a combination of morphological characters. Here, we follow the concept of Largent (1994) and Baroni & Halling (2000) for *Inocephalus* (Noordel.) P.D. Orton based on these morphological features: a mycenoid or collybioid habit, a non-glabrous innately fibrillose to squamulose pileus, an entangled pileipellis often nearly a trichoderm on the disc, a pileipellis that intergrades imperceptively with the pileal trama, abundant oleiferous hyphae and lipoid bodies, cheilocystidia and/or pseudocystidia, and basidiospores with 4–7 distinct angles, some of which are cuboid.

Cuboid basidiospores, unique to the *Entolomataceae,* have interested investigators ever since *Entoloma virescens* (Sacc.) E. Horak ex Courtec. was described from the Bonin Islands in 1857 (Horak 1976). Their history, distribution, systematics, taxonomic significance, and development within the *Entolomataceae* have been thoroughly reviewed (Baroni & Halling 2000; Eyssartier et al. 2001; Horak 1976, 1977; Pegler & Young 1978; Romagnesi 1941). In Australia, two species with a cuboid spore-type have been reported: *Inocephalus virescens* (Sacc.) Largent & Abell-Davis (≡ *E. virescens*) (Young 2005, Largent & Abell-Davis 2011) and *E. procerum* G. Stev. (May & Wood 1997, Ratkowsky & Gates 2002, Noordeloos & Gates 2012). Herein, we describe three new species with cuboid basidiospores: *Inocephalus plicatus*, *I. hypipamee*, and *I. parvisporus*.

Materials & methods

Macromorphological and micromorphological features

Specimens were collected during February–April 2009–11 from various localities within the Wet Tropics Bioregion throughout northeastern Queensland and during April 2010–11 from temperate rainforests in central New South Wales. General and technical names indicated by quotations marks in the descriptions are from the Colour Diagrams Section of Kornerup & Wanscher (1978: 196–225).

The factors determined from mathematical analyses in the descriptions include: the arithmetic means ($\bar{x}$) of spore lengths and spore widths ± standard deviation in n objects measured; the quotient of spore length by spore width (E) indicated as a range variation in n objects measured; the mean of E-values (Q) ± standard deviations. The sample size (n) is equal to the total number of microscopic structures measured (x) and the number of basidiomata studied (y) and is given in the format n = x/y. The Q values are used to define general basidiospore shape with the following types recognized: isodiametric with Q = 1.0–1.15; subisodiametric with Q = 1.16–1.27; heterodiametric Q >1.27 (Largent 1994). Details of macroscopic and microscopic features and measurements were described in a previous paper (Largent & Abell-Davis 2011).

All collections for New South Wales cited in the 'Additional collections examined' were deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR), while collections made in Queensland were split with duplicates deposited in the Australian Tropical Herbarium (CNS) and The Queensland Herbarium (BRI). All holotype and isotype collections are deposited in the herbaria designated using acronyms from Thiers (2012).

DNA sequences

The details of protocols for DNA extractions, PCR amplification of partial sequences of the mitochondrial small subunit of the ribosomal DNA (mtSSU), variable domains (D1, D2) of the nuclear large subunit (LSU) and sequencing protocols are described in previous publications (Largent et al. 2011a, b). PCR primers used for amplification of the second largest subunit of the RNA polymerase gene (RPB2) from *Inocephalus* were performed with either of one of two forward primers, rpb2-EntF1 (5′ to 3′– GAA GGT CAA GCT TGY GGT C) or rpb2-EntF2 (5′ to 3′– GAA GGY CAR GCY TGY GGT C) or with either one of two reverse primers, rpb2-EntR3 (TGG ATY TCR CAR TGC GTC CA) or rpb2-EntR5 (TGR ATY TCR CAR TGG GTC CA). PCR amplifications of the RPB2 gene were performed in 25 µL reactions containing 1× GoTaq Flexi Buffer (Promega, Madison WI USA), 2 mM $MgCl_2$, 0.2 mM dNTPs, 500 nM forward and reverse primers, 0.2 mg/mL bovine serum albumin, 1 M betaine, 0.025 U Taq polymerase, and 1–3 µL of undiluted template DNA. PCR cycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 30 sec, 52°C or 54°C or 56°C for 1 min, and 72°C for 1 min, followed by a final extension of 72°C for 10 min. Forward and reverse sequences for each locus were generated on an Applied Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University following the protocols outlined in Largent et al. (2011a, b).

Results

We obtained partial sequences for the mitochondrial small subunit rRNA gene (mtSSU), nuclear large subunit (LSU), and the second largest RPB2 subunit for a subset of collections of *I. plicatus* and *I. hypipamee* (Table 1). Notes on the morphology of the basidiomata exist for seven *I. plicatus* collections and

Table 1. *Inocephalus* specimens included in this study.

Species	Collection Identifier	GenBank Accession Numbers		
		mtSSU	LSU	RPB2
I. hypipamee	DL Largent 9649	JQ624602	JQ624607	-
	DL Largent 9981	JQ624603	JQ624608	-
	DL Largent 10071	JQ624604	JQ624609	JQ624616
I. plicatus	DL Largent 9691	JQ624605	JQ624610	JQ624617
	DL Largent 9885	-	JQ624611	JQ624618
	DL Largent 10083	-	JQ624612	JQ624619
	DL Largent 10091	-	JQ624613	JQ624620
	DL Largent 10096	-	-	JQ624621
	DL Largent 10124	-	JQ624614	JQ624622
	DL Largent 10216	JQ624606	JQ624615	JQ624623

the three *I. hypipamee* collections with sequences. These sequences were used to formulate the descriptions and develop the diagnostic characters for both species.

Sequences of *I. parvisporus* were omitted after several attempts to sequence PCR products from the three loci failed.

Taxonomy

Inocephalus plicatus Largent, **sp. nov.** PLATES 1–2

MYCOBANK MB 564924

Differs from *Inocephalus pseudomurrayi* by its larger basidiomata, yellow pileus maturing to plicate-striate and yellowish orange, pungent odor, latently unpleasant taste, and pseudocystidia on lamellar edge and face.

TYPE — Australia, New South Wales, central Hunter District, Strickland State Forest, Lower Parking Lot Track, within 20m of 33°22′47″S 151°19′32″E, 16 April 2010, DL Largent 9885 (**holotype** DAR).

ETYMOLOGY — from the Latin *plicatus* referring to the pleated (plicate) pileal surface.

PILEUS 17–40 mm broad, 5–8 mm high, conic to convex at first, becoming broadly convex to broadly campanulate with maturity, with a rounded to mammillate umbo, glabrous or atomate to the eye, or under 4× or 20× magnification, minutely appressed-fibrillose except for the minutely roughened center, dull, opaque, not hygrophanous, at first even but with maturity quickly becoming striate and then plicate-striate from the margin to near the center, at first pale yellow to light yellow; (2-3A5, 3-4A4), often with a slightly darker center (3A6, 4A5-4B6, 4A5-6) becoming greyish yellow to greyish orange when picked, drying or fading (4-5A-B3-4, 5B4-5, 7E-F8); margin decurved at all times, entire and with age becoming broadly lobed and crenulate and finally eroded. TASTE mild at first and then latently bitter to unpleasant. ODOR mild and then pungent. LAMELLAE 6–20 mm long, adnexed, subdistant, moderately broad (3–8 mm), pale yellow at first (3A3) becoming orange white to near pale orange with spore maturity (4-5A2-3, 4A3-4, 5A3-4, 5A5-6); margin smooth and concolorous to the eye, cystidiate under 4× or 20× magnification; 3 lamellulae (2 medium short; 1 medium long) in two layers between the lamellae. STIPE 35–85 mm long, 3–5 mm broad at apex, 3.5–8 mm broad at base, at times flattened and longitudinally grooved and then 3 × 4.5 mm broad at apex, 4 × 5 mm broad at base, typically clavate, rarely twisted, glabrous except minutely roughened to pruinose at the apex, pale yellow to light yellow (2A4 to 3-4A2-4) darkening to pale orange (5A3-5, 5B4) and/or discoloring to light brown (4-5D7, 5D7) stuffed at first, becoming hollow, sturdy; basal tomentum scarce to absent. BRUISING REACTIONS pileus frequently staining brownish orange (6C8) to dark brown with a reddish orange tint.

Plate 1. *Inocephalus plicatus* (DLL 10091). A: Basidiomata (2.5×); B: Pileus surface (2×); C: Stipe apex (8×).

Basidiospores in profile view versiform, cuboid, rhomboid, and at times rectangular, often with one angle enlarged, rarely 3-angled and then trapezoid-like, sometimes 5-angled, isodiametric to heterodiametric in dorsal or ventral view, 4–5-angled and isodiametric in polar view, 7.7–11.9 × 6.6–10.4 µm (x = 9.6 ± 0.9 × 8.5 ± 0.8 µm; E = 1.0–1.4; Q = 1.2 ± 0.1 (subisodiametric); n = 169/7). Basidia typically long and clavate, tapered to the base, the base typically enlarged, 40.5–73.2 × 9.5–18.1 µm (x = 55.5 ± 6.9 × 12.4 ± 1.5 µm; E = 3.2–7.4; Q = 4.5 ± 0.8; n = 53/6); 4-sterigmate, sterigma 3.7–6.2 µm; base of basidium 1.9–5.2 µm. Aborted basidia absent. Cheilocystidia typically forming a sterile layer, at times scattered, colorless to opaque, clavate to cylindro-clavate eventually becoming strangulated to somewhat contorted, tapering to the apex, 66.5–144.4 × 4.8–12.2 µm (n = 14/2). Pseudocystidia scattered to abundant on the lamellar edge and face, filled with granular refractive material yellowish in 3% KOH, cylindro-clavate, typically contorted, 21.8–87.3 × 3.2–9.1 µm (n = 29/4). Hyphae of the lamellar trama subparallel and quite long, 136–1119 × 3.7–22.7 µm (n = 37/5). Pileipellis an entangled, semi-erect layer of hyphae, entirely erect at first, with maturity remaining so in the center but a cutis elsewhere. Pileocystidia cylindro-clavate, 66.5–144.4 × 4.9–21.5 µm (n = 22/3). Hyphae of the pileal trama quite long, 43.6–507.0 × 6.1–26.9 µm (n = 11/3). Stipitipellis at the apex an entangled layer of semi-erect caulocystidia. Caulocystidia cylindro-clavate, similar to the pileocystidia, 37.6–167.6 × 4.6–11.2 µm (n = 30/5). Hyphae of the stipe trama not studied. Lipoid globules abundant in all tissues and obscuring all microscopic features when viewed in 3% KOH. Oleiferous hyphae abundant in the lamellar trama, in the subhymenium, and the pileal trama. Brilliant granules absent. Pigmentation cytoplasmic and yellowish in the hyphae of the pileipellis and stipitipellis in 3% KOH. Clamp connections present in all tissues.

Ecology and distribution — Solitary, scattered, or gregarious in sandy organic rich soil, in deep leaf humus or protected by logs and woody debris, warm temperate gallery rainforest near *Ceratopetalum apetalum* D. Don, *Backhousia myrtifolia* Hook., and *Syncarpia glomulifera* (Sm.) Nied. (Strickland State Forest, New South Wales). In other localities, found in warm, temperate,

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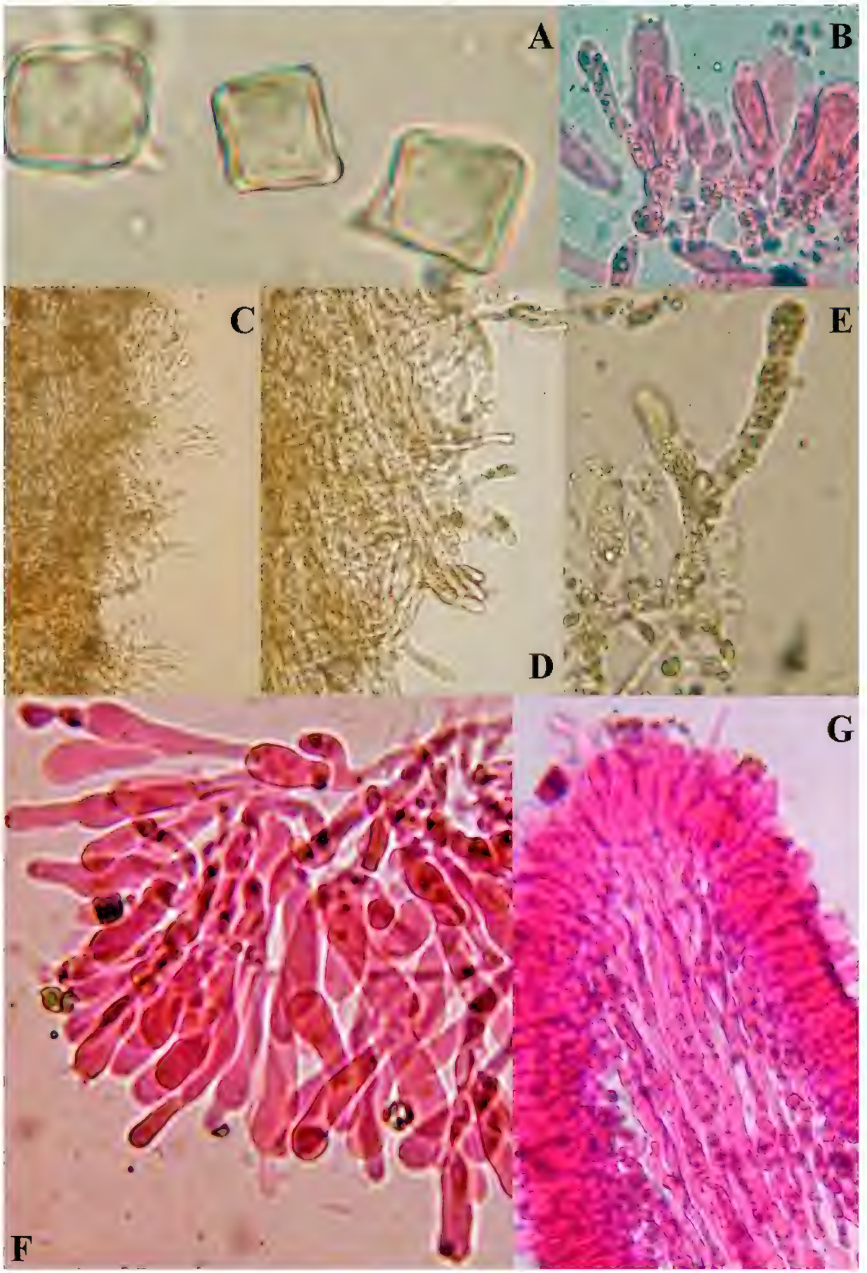
Plate 2. *Inocephalus plicatus*. A: 4-angled basidiospores (2500×), rectangular in profile view (left), nearly square in dorsi-ventral view (center), and becoming rhomboid in profile view (right) (DLL 10091); B: Pseudocystidium to left of basidium (2000×) (SSF 12); C: Stipitipellis with cylindro-clavate caulocystidia (50×) (SSF 12); D: Pileipellis with clavate to cylindro-clavate pileocystidia (125×) (SSF 12); E: Pseudocystidium from lamellar face (450×) (DLL 10091); F: Strangulated to somewhat contorted cheilocystidia, 2-sterigmate basidium (lower left corner), and oleiferous hyphae (darker) (350×) (DLL 9885, holotype); G: Section of lamellae with abundant oleiferous hyphae, lipoid globules, and showing origin of pseudocystidium (100×) (DLL 9885, holotype).


A
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subtropical gallery rainforests (New South Wales) and wet tropical rainforests (northeastern Queensland).

Additional collections examined — **AUSTRALIA. New South Wales**, central Hunter District, Barrington Tops National Park, Jerusalem Creek Track, 25 April 2010, S Moore 59; Strickland State Forest, Lower Parking Lot, 33°22′47″S 151°19′27″E, 23 March 2009, SSF07, SSF12; 19 April 2010, DL Largent 9903; 33°22′47″S 151°19′31″E, 14 April 2011, DL Largent 10091, 10096; 33°22′48″S 151°19′28″E, 19 April 2011, DL Largent 10124; 33°22′47″S 151°19′33″E, 28 April 2011, DL Largent 10165; 33°22′47″S 151°19′29″E, 7 May 2011, DL Largent 10216; Watagans National Park, Boardinghouse Dam Area, 32°59′56″S 151°24′19″E, 12 April 2011, DL Largent 10083. **Queensland**, Cook Region, Herberton National Park, 17°27′07.0″S 145°28′32.8″E, 24 March 2009, DL Largent 9691; 17°27′14.8″S 146°28′34.2″E, 16 March 2010, DL Largent 9786; Mt. Lewis National Park, 16°16′10.1″S 147°16′11.0″E, 20 March 2010, DL Largent 9803; Mossman Gorge National Park, 16°28′16.9″S 145°19′51.6″E, 25 February 2010, DL Largent 9741, 9742.

Comments — *Inocephalus plicatus* is distinguished from other *Inocephalus* taxa by the following set of diagnostic features: yellow basidiomata when young with a pileus that is greyish yellow to greyish orange, plicate-striate and mammillate umbonate at maturity and that stains brownish orange or darker with handling; pileus tissue producing a latently bitter to unpleasant taste and a latently pungent odor; mostly cuboid or rhomboid basidiospores averaging ≤10 × 9.0 µm; contorted and refractive pseudocystidia on lamellar face and edge as well as abundant, contorted or strangulated and opaque cheilocystidia; a pruinose stipe apex composed of cylindro-clavate caulocystidia; abundant oleiferous hyphae in the lamellar and pileal trama; and abundant clamps.

Morphologically, *I. plicatus* resembles *Entoloma pseudomurrayi* Eyssart. et al., *E. cuspidatum* Sacc., and *I. murrayi* (Berk. & M.A. Curtis) Rutter & Watling (≡ *E. murrayi* (Berk. & M.A. Curtis) Sacc.).

Entoloma pseudomurrayi from New Caledonia shares many similar morphological features with *I. plicatus* but is differentiated by the smaller basidiomata, the citrine yellow, non-plicate, non-staining pileus that does not darken to greyish yellow or greyish orange, the mild odor and taste, the lanceolate to fusiform and moniliform cheilocystidia, and pseudocystidia found only on the lamellar edge (Eyssartier et al. 2010).

Entoloma cuspidatum (≡ *Agaricus cuspidatus* Peck, nom. illegit., non *A. cuspidatus* Bolton) is morphologically differentiated from *I. plicatus* by its conic to campanulate, cuspidate, pale yellow, non-striate pileus and basidiospores measuring 9–13 × 8–12 (–12.5) µm. The basionym of *Inocephalus murrayi* (≡ *Agaricus murrayi* Berk. & M.A. Curtis) is morphologically differentiated from *I. plicatus* by its non-plicate pileus that turns reddish brown on drying and basidiospores measuring 9–11 × 9.5–11 µm.

The synonomy of *Entoloma cuspidatum* and *Inocephalus murrayi*

Entoloma cuspidatum is considered a synonym of *I. murrayi* by Baroni & Halling (2000) and of *E. murrayi* by Hesler (1963, 1967), Horak (1976, 1980), and Singer (1942). This synonymy should be re-evaluated because of the following discrepancies.

Peck's (1872: 64–65) protologue description of *A. cuspidatus* differs from that of *A. murrayi* (Berkeley & Curtis 1859: 289). *Agaricus cuspidatus* has a conic to campanulate, non-striate pileus pale yellow throughout, an elongated papilla or cusp, and basidiospores 1/2000 inch (= 12.7 µm) in diameter. *Agaricus murrayi* has a convex regularly striate pileus that is straw yellow turning rich red brown when dry and basidiospores 1/2500 inch (= 10.16 µm) in diameter. Using these features, Peck considered the two species related but different (Noordeloos 2008: 96).

Studies of the holotype collections indicate the basidiospores of *A. murrayi* measure 9–11 × 9.5–11 µm (Singer 1942) and are indeed a little smaller than those measured for *A. cuspidatus* Peck (9–13 × 8–12 µm, Hesler 1963; 11–13.5 (–14) × (9.5–) 10–11.5 (–12.5) µm, Baroni pers. comm.; 9–11.7 × 8.0–11.7 µm, Noordeloos 2008).

Australasian species morphologically similar to *Inocephalus plicatus*

Several other species from Australasia morphologically resemble *I. plicatus* in yellow coloration and cuboid basidiospores. *Entoloma pallidoflavum* (Henn. & E. Nyman) E. Horak from Java, Borneo, and New Guinea is differentiated by the larger pileus (30–80 mm) and stipe (≤130 mm long), an indistinctive or radish-like odor, and coarsely dentate-serrate lamellae. *Entoloma albogracile* E. Horak from Papua New Guinea and *E. avilanum* (Dennis) E. Horak from Venezuela have tetrahedral-cuboid basidiospores. *Entoloma gracilius* E. Horak from Papua New Guinea has smaller basidiospores (5.5–7.0 µm; Horak 1976). *Inocephalus luteus* (Peck) T.J. Baroni (≡ *Entoloma luteum* Peck) from eastern North America is morphologically differentiated by the smoky yellow, at times greenish yellow, distinctly fibrillose pileus as typified by the protologue description and illustration (Baroni, pers. comm.).

Inocephalus hypipamee Largent, **sp. nov.** PLATES 3–4

MYCOBANK MB 564925

Differs from *Entoloma flavotinctum* by its larger basidiomata, non-hygrophanous pileus, white then yellowish white stipe, white context, and larger basidiospores.

TYPE — Australia. Queensland, Cook Region, Mt. Hypipamee National Park, within 20 m of 17°25′35.2″S 145°29′14.6″E, 25 March 2011, DL Largent 10071 (**holotype** BRI, **isotype** CNS).

ETYMOLOGY — from the Australian Aborigine *hypipamee,* referring to the frequent occurrence of this species in Mt. Hypipamee National Park.

Pileus 9–70 mm broad, 4–23 mm high, when young narrowly parabolic, conic, or convex, entirely and densely matted-tomentose to tomentulose, quickly developing an acute to broad umbo; upon expansion and maturity becoming broadly conic, broadly convex, campanulate-convex, or campanulate, always remaining densely matted-fibrillose to tomentulose on the apex and umbo but becoming densely appressed-squamulose elsewhere, eventually becoming matted-fibrillose-squamulose and somewhat rimulose at or near the margin, overly mature specimens becoming fibrillose and rimose everywhere except on or near the umbo; when very young or in basidiomata protected by overhanging logs, the fibrils, hairs, and tomentum greyish orange to brownish orange (6B-C2-3) on and around the umbo and white to orange white (6A-B1-2) elsewhere, upon exposure and in young exposed basidiomata brown to dark brown (6F4-5) everywhere, in maturing specimens remaining so on or near the umbo, elsewhere fading to a lighter brown (between 5E3-4 and 6E3-4) then becoming brownish orange (between 5C-D3-4 and 6C-D3-4) from the margin to the umbo, in very old specimens the fibrils become rimose and thus the orange white to greyish orange (6A-B2-3) background color becomes visible; surface opaque (not hygrophanous, not translucent-striate); margin decurved and entire, eventually becoming plane or uplifted, fibrillose, crenulate, and eroded; context up to 1.25 mm above the stipe, <1 mm midway to margin, and nearly non-existent at the margin, white to off-white. Taste mild becoming, at most, latently slightly unpleasant. Odor mild or indistinct. Lamellae 6–32 mm long, 1–9 mm deep, when young yellowish white (3A2) then very pale yellow (4A3) and with spore maturity between orange white and pale orange (5A2 or 5A3), narrowly adnate or broadly adnexed at all times but often with a decurrent tooth, at first close to crowded and narrow then subdistant and moderately broad to sigmoid; margin smooth and concolorous to the eye but under the dissecting microscope abundantly cystidiate; 3 lamellulae/2 lamellae. Stipe 18–68 mm long, 1–7 mm broad at apex, 2–8 mm broad at base, equal in young specimens, but with maturity typically enlarged at the base, white or off-white (3A2) becoming yellowish white (4A2) with age, to the eye glabrous except pruinose at the apex, under the dissecting microscope suggestively appressed-fibrillose, hollow but solid or sturdy; basal tomentum scarce to absent; context unchanging when cut. Bruising reactions slightly brownish on the stipe.

Basidiospores in profile view typically cuboid or rectangular, sometimes 5-angled, isodiametric to heterodiametric in profile and dorsiventral views, isodiametric in polar view, 7.1–10.4 × 6.2–8.6 µm (x = 8.8 ± 0.8 × 7.4 ± 0.6 µm; E = 0.9–1.4; Q = 1.2 ± 0.1 (subisodiametric); n = 58/2). Basidia mostly clavate, typically long and tapered to the base, 37.9–49.3 × 9.0–12.0 µm (x = 43.2 ± 3.24 × 10.1 ± 0.79; E = 3.4–5.3; Q = 4.1 ± 0.5; n = 20/2); 4-sterigmate, sterigma 2.5–4.1

Plate 3. *Inocephalus hypipamee*. A: Basidiomata stature (2×) (DLL 10035); B: Pileus surface, young (left), and mature (right) (2×) (DLL 10035); C: Lamellae and lamellulae (2×) (DLL 9999).

µm long; base of basidia 3.5–5.4 µm wide. Aborted basidia rare to scattered, embedded in the hymenium. Hymenial cystidia abundant and obvious, present as Pseudocystidia, typically filled with a yellowish oily material, becoming reddish brown in 3% KOH, those on the lamellar edge clavate to obclavate or fusoid, 38.3–46.2 × 4.4–12.8 µm (n = 7/1); those on the lamellar face similar in shape but longer than those on the lamellar edge, originating from the outer layers of the lamellar trama, 47.6–106.5 × 5.0–19.5 µm (x = 76.6 ± 18.4 × 12.7 ± 3.7 µm; E = 4.3–9.6; Q = 6.3 ± 1.6; n = 14/2). Hyphae of the lamellar trama subparallel, moderately long to long, 107.7–582.3 × 4.2–14.7 µm (n = 8/2). Pileipellis in fresh specimens composed of semi-erect and laterally agglutinated hyphae with cylindro-clavate to clavate terminal cells, in dried specimens also composed of entangled and laterally agglutinated hyphae but with cylindro-clavate terminal cells often collapsed on top of clavate ones. Pileocystidia cylindro-clavate or clavate 30.5–51.6 × 5.9–14.6 µm (n = 6/1). Hyphae of the pileal trama above the lamellar trama 55.0–173.8 × 9.8–15.0 µm (n = 9/1). Stipitipellis a cutis except at the apex with clusters of basidioles or small clavate caulocystidia. Caulocystidia 31.0–41.2 × 7.1–10.7 µm (n = 3/1). Hyphae of the stipe trama parallel, 55.0–188.0 × 9.8–26.3 µm (n = 9/1). Lipoid globules abundant in all tissues, often obscuring other microscopic features. Oleiferous hyphae abundant throughout the lamellar and pileal trama. Brilliant granules absent. Pigmentation composed of abundant dark brown, cytoplasmic plaque-like areas in the hyphae of the pileipellis, absent elsewhere. Clamp connections absent in all tissues.

Ecology and distribution — Typically solitary but at times gregarious with clusters of 2-4 basidiomata, in granitic soil and leaves beneath various species of *Lauraceae* and *Myrtaceae* in a complex notophyll vine forest of cloudy and moist to wet highlands. In Mt. Hypipamee National Park, *I. hypipamee* produced 50–100 basidiomata throughout the same forested slope above Dinner Falls from 16 February to 5 April 2011.

Additional collections examined: **AUSTRALIA. Queensland**, Cook Region, Mt. Hypipamee National Park, 17°25′35.4S″ 145°29′12.4″E, 14 March 2009, DL Largent 9649; 17°25′34.5″S 145°29′16.6″E, 16 February 2011, DL Largent 9981, 9981A, 9982; 17°25′35.4″S 145°29′10.9″E, DL Largent 9984; 17°25′58.6″S 145°29′27.4″E, 21 February 2011, DL Largent 9988; 17°25′34.8″S 145°29′17.0″E, 28 February 2011, DL Largent 9992; 17°25′37.1″S 145°29′15.2″E, 5 March 2011, DL Largent 9998; 17°25′35.2″S 145°29′14.6″E, DL Largent 9999; 17°25′36.7″S 145°29′15.7″E, 17 March 2011, DL Largent 10035; 17°25′35.2″S 145°29′14.6″E, 29 March 2011, DL Largent 10074; 17°25′36.7″S 145°29′15.7″E, 5 April 2011, DL Largent 10081; Mt. Lewis National Park, 16°34′15.3″S 145°15′54.9″E, 20 March 2010, DL Largent 9804.

Comments — *Inocephalus hypipamee* is diagnosed by the mammillate, broadly conic to broadly convex to convex-campanulate pileus with dark brown tomentum in the center and dark brown fibrils and squamules elsewhere, the

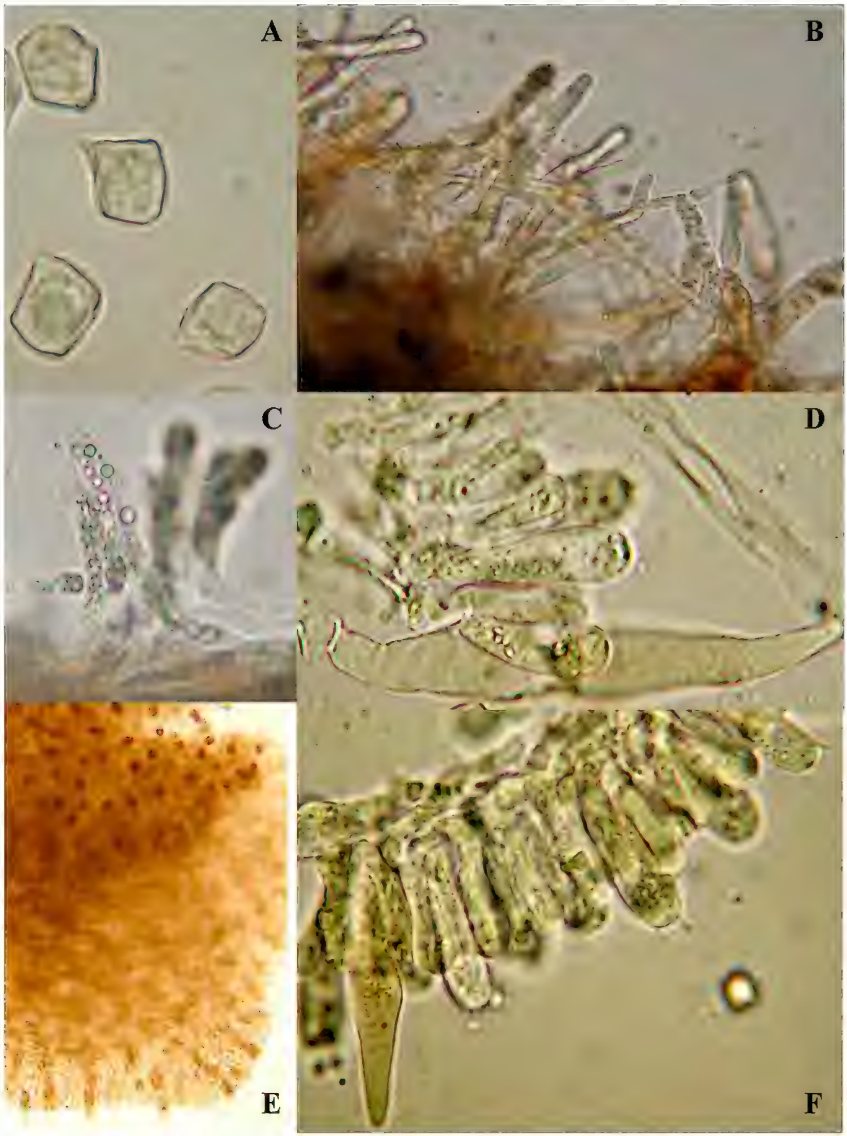

Plate 4. *Inocephalus hypipamee*. A: Basidiospores in profile view (1700×) (DLL 9649); B: Pileipellis composed of entangled hyphae with cylindro-clavate pileocystidia (1000×) (DLL 9988); C: Caulocystidia at apex of stipe (1000×) (DLL 9988); D: narrowly obclavate pseudocystidium originating from outer portion of lamellar trama, nearby basidioles (in focus), and mature basidium (out of focus) (150×) (DLL 9649); E: pseudocystidia on face of lamella staining reddish-brown in 3% KOH (1000×) (DLL 9988); F: Obclavate pseudocystidium, and basidia (1000×) (DLL 9649).

white to off-white lamellae and stipe, the cuboid to rectangular, subisodiametric basidiospores measuring 7.1–10.4 × 6.2–8.6 µm, the clavate to obclavate or fusoid pseudocystidia found on the lamellar edge and face, and the absence of clamp connections.

Because of its brown pileus, white lamellae and stipe, cuboid basidiospores, pseudocystidia and absence of clamp connections, *E. flavotinctum* E. Horak & Corner from Brazil is the only species remotely similar to *I. hypipamee*. *Entoloma flavotinctum* is morphologically differentiated by its smaller basidiomata, hygrophanous pileus, a stipe that becomes yellowish or greenish ochraceous, a white context that turns yellowish on cutting and bruising, and smaller quadrate basidiospores (6–8(–9) µm; Horak 1982).

Inocephalus parvisporus Largent, **sp. nov.** PLATES 5–6

MYCOBANK MB 564926

Differs from *Entoloma neocaledonicum* by the *Leptonia*-like pileus with a small broad umbo in a depression, white lamellae, longer cheilocystidia, and smaller basidiospores.

TYPE — Australia, Queensland, Cook Region, Danbulla National Park, Kauri Creek Track, within 20m of 17°07′42.3″S 145°25′55.0″E, 25 March 2009, DL Largent 9829 (**holotype** BRI, **isotype** CNS).

ETYMOLOGY —from the Latin words *parvi* + *sporus*, referring to the small basidiospore size.

PILEUS 10–32 mm broad, 5–15 mm high, when young dark brown with a reddish tinge (near 8F4) on the disc and ornamentations with a slightly lighter dark brown (8E4) background, parabolic or campanulate-convex, or convex, entirely minutely tomentulose, and typically with a small, broad umbo and an even, incurved margin, when maturing and expanding remaining dark brown (8F4) on the disc becoming reddish white to pale red (7A-B2-3) elsewhere, becoming broadly convex to broadly campanulate-truncate with a depression developing, the umbo often disappearing, remaining tomentulose in the center becoming minutely squamulose to appressed-fibrillose towards the margin, and appressed-fibrillose at the margin, with the margin becoming decurved, crenulate-lobed and then eroded, typically opaque, at times becoming hygrophanous in the disc, not striate nor translucent-striate, dull when dry, shiny when moist. TASTE indistinct or mild. ODOR indistinct or mild. LAMELLAE 4–19 mm long, 1.75–5 mm deep, narrow then quickly moderately broad, adnate, close to subdistant, white when young with spore maturity remaining light on the edge but becoming flesh-colored elsewhere; margin smooth and concolorous, entire at all times; up to 5 lamellulae between lamellae, in 3 tiers (2 short, 2 medium, 1 medium long). STIPE 27–45 mm long, 1.25–4 mm broad, equal, pruinose at the apex, glabrous with hygrophanous streaks elsewhere, white (6A1) but bruising reddish grey (8B-C2), hollow with some solidity

Plate 5. *Inocephalus parvisporus* (DLL 9829, holotype). A: Basidiomata stature (2×); B: Lamellae and pruinose stipe apex (2×); C: Pileus surface (2×).

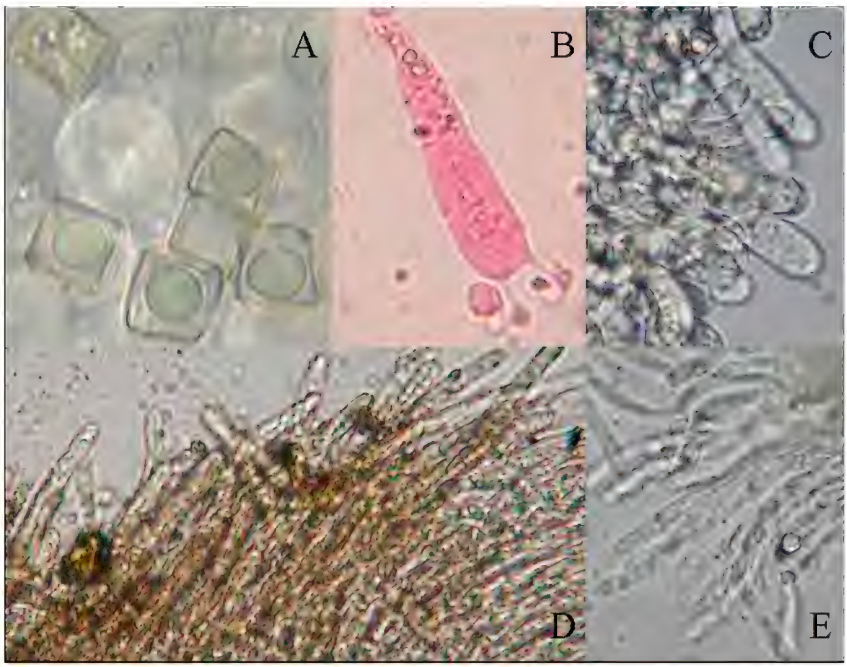

Plate 6. *Inocephalus parvisporus* (DLL 9829, holotype). A: Basidiospores (2700×); B: Basidium (1000×); C: Cylindro-clavate to clavate cheilocystidia (300×); D: Pileipellis just off disc with cylindro-clavate pileocystidia (200×); E: Cylindro-clavate caulocystidia at stipe apex (900×).

but ultimately becoming fragile; basal tomentum moderate becoming scarce. Bruising reactions absent.

Basidiospores cuboid, square to rectangular, rarely rhomboidal in profile view, 5.3–8.1 × 4.5–7.1 µm (x = 6.5 ± 0.6 × 5.6 ± 0.7 µm; E = 1.0–1.5; Q = 1.2 ± 0.1 (subisodiametric); n = 30/1). Basidia clavate and tapered, 33.5–41.4 × 10.4–13.1 µm (x = 38.9 ± 2.3 × 11.2 ± 0.9 µm; E = 2.9–3.9; Q = 3.5 ± 0.3; n = 9/1), 4-sterigmate. Aborted basidia absent. Cheilocystidia cylindro-clavate to clavate, abundant, portions of some lamellar edge completely sterile, similar in size to the caulocystidia. Pleurocystidia and Pseudocystidia absent. Hyphae of the lamellar trama subparallel 92.2–398.5 × 4.4–10.5 µm (n = 6/1). Pileipellis a trichodermal palisade, with the hyphae erect but entangled on the disc, semi-erect and entangled towards the margin, and repent near and at the margin; Pileocystidia 48.3–148.6 × 4.9–9.3 µm (n = 6/1), long and cylindro-clavate, resembling the cheilocystidia. Hyphae of the pileal trama 357.8–491.3 × 13.4–16.6 µm (n = 3/1). Stipitipellis with abundant cylindro-

clavate cystidioid caulocystidia at the apex, a cutis elsewhere. Caulocystidia clavate, similar to the cheilocystidia, 52.4–66.5 × 4.6–7.2 µm (n = 4/1). Hyphae of the stipe trama not studied. Lipoid globules abundant, particularly in the lamellar and pileal trama. Oleiferous hyphae abundant, particularly in the lamellar and pileal trama. Brilliant granules absent. Pigmentation with dark brown cytoplasmic particles in the hyphae of the pileipellis, absent elsewhere. Clamp connections absent in all tissues.

Ecology and distribution — Scattered to gregarious on leaves in humus along side of Kauri Creek Track, near the end of the track as it drops down to Kauri Creek, Danbulla National Park; late March; known only from the type locality.

Comments — Unique features of *Inocephalus parvisporus* include its *Leptonia*-like habit and basidiomata with dark brown, umbonate, opaque pilei with slight reddish tones, the small basidiospores, the presence of cheilocystidia, small cuboid basidiospores, and no clamps. In the field, it resembles a species of *Leptonia* (Fr.) P. Kumm., although the abundant oleiferous hyphae, abundant lipoid bodies, and cuboid basidiospores suggest *Inocephalus*.

Because of their brown basidiomata and cuboid basidiospores, *Entoloma griseoalbum* E. Horak from Papua New Guinea and *E. neocaledonicum* E. Horak from New Caledonia share similar morphological features with *I. parvisporus*. However, both *E. neocaledonicum* and *E. griseoalbum* are morphologically differentiated by their mycenoid basidiomata with a conical pileus. In addition, *E. griseoalbum* has a grey brown striate pileus and clavate cheilocystidia often with finger-like protrusions, and *E. neocaledonicum* has pale wax yellow lamellae, broadly clavate cheilocystidia, larger basidiospores, and clamp connections (Horak 1976, 1977).

Acknowledgments

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New *Lecanora*, *Lecidea*, *Melaspilea*, *Placynthium*, and *Verrucaria* records for Turkey and Asia

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Abstract — Five species of lichenized fungi – (*Lecanora invadens*, *Lecidea promiscua*, *Melaspilea interjecta*, *Placynthium garovaglioi*, and *Verrucaria bryoctona*) – are reported as new to Turkey. Three of them, *M. interjecta*, *P. garovaglioi*, and *V. bryoctona*, are also new to Asia. Descriptions are presented, including geographic distribution, substrate, chemistry, and comparisons with morphologically similar taxa.

Key words — *Ascomycota*, biodiversity, Iğdır, Van, Burdur

Introduction

The lichen flora of Turkey is still incompletely known, as it is in many parts of the world. Although many recent studies contributed to the lichen flora of Turkey (Halıcı & Cansaran-Duman 2008, Karagöz et al. 2011, Kınalıoğlu 2010, Halıcı et al. 2010a,b, Osyczka et al. 2011, Candan & Halıcı 2011, Oran & Öztürk 2012, Senkardeşler 2010, Yazıcı & Aslan 2009, Yazıcı et al. 2008, 2010a,b, 2011a,b), there are more unexplored regions than explored ones in the country. Therefore, more studies are needed to complete the lichen flora of Turkey.

Here we report five new records for Turkey, of which three are new for Asia, based on our fieldwork in the regions of Van, Iğdır and Burdur in eastern Turkey.

The Van region has a climate characterized by cold and snowy winters and hot dry summers, with a temperature range of –26.9–36.0 °C, a mean annual rainfall of around 370–570 mm, and mean annual humidity of 51–66% (Akman 1999). The examined area is dominated by steppe vegetation and lacks trees completely. Three localities were surveyed: Kapı village in Saray district near the Iranian border is an open windswept area with gently sloping rocky terrain;

the Çatak district is a partly well lit high elevation sloped terrain with a waterfall and humid rocks; and Bahçesaray, at the border of the Van district, follows the road through a well-lit windswept weakly sloping land with a stream and humid rocks (Baytop & Denizci 1963).

The climate of the Iğdır region is similar to Van, with a mean annual temperature of 11.6 °C, mean annual rainfall of 257.6 mm, and average humidity of 63%, Precipitation is generally moderate in the summers and heavy during winter (Akman 1999). The Iğdır region is dominated by steppe with few forested areas, although *Elaeagnus, Populus*, *Prunus*, *Pyrus,* and *Salix* trees are seen occasionally in the visited locality, Kula village (Tuzluca) (Baytop & Denizci 1963). Kula is a well-lit, high elevation, windswept, treeless area with gently sloping terrain containing streams, grass, and many calcareous and siliceous rocks.

Burdur has a continental Mediterranean climate with cold, snowy winters and very hot, long, dry summers with a mean annual temperature of 15°C and the temperature range of –16–39 °C, mean annual rainfall of about 468 mm, and average humidity of 51.2% (Akman 1999). The visited area, Bucak district, is mountainous with much forest dominated by *Abies*, *Cedrus*, *Ficus*, *Fraxinus*, *Juniperus*, *Olea*, *Pinus*, *Prunus*, *Quercus*, *Rhus*, and *Pistacia* alternating with streams and lakes (Baytop & Denizci 1963).

Materials & methods

Lichens were collected from five localities in the districts Iğdır, Burdur and Van in Turkey in July 2009. Air-dried samples were examined with a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i compound light microscope. For the identifications relevant keys consulted (Czeika & Czeika 2007, Hertel 2006, Magnusson 1952, Orange 1991, Sliwa 2007, Smith et al. 2009). Vouchers are stored in the Herbarium of the Biology Department, Karadeniz Technical University, Trabzon, Turkey (KTUB) and in the Herbarium of the Biology Department, Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey (ATAKKEF). The descriptions are based on Turkish specimens and supplemented by data from the indicated literature.

Species recorded

Lecanora invadens H. Magn., Lichens Central Asia: 87. 1940.

Thallus crustose, ± superficial or evanescent, indistinct, to 0.6 cm in diam., more evident around apothecia, ± grey to greenish grey, often with distinct bluish pigment. Apothecia mostly clustered in groups, sessile, or constricted at the base and raised, ± flat when mature, to 0.6 mm in diam.; disc plane to slightly convex, ± smooth, dark brown to slightly black and slightly pruinose, rarely epruinose; margin level ± with the disc ± rough, entire, pruinose, paler than the thallus, white. Amphithecium 155–175 µm thick, corticate, with algae mostly dense below the cortex; cortex mostly delimited, slightly thicker at the

base, 16–55 µm thick, composed of adglutinated hyphae, thin and bluish-green at the very top of the margin; epithecium yellowish-brown, granular; granules usually sparse, between the paraphysis tips; hymenium hyaline, 50–65 µm high; hypothecium hyaline or yellow, 60–100 µm high. Paraphyses simple or branched at the tips, slender or ± thickened, ± pigmented at the tips. Asci clavate, 8-spored; ascospores hyaline, simple, ± broadly ellipsoid, 9–12 × 6–8 µm. Pycnidia absent. Apothecial margin K± yellow, C–, KC± yellow, P–; disc K± yellow, C–, KC± yellow, P–.

Lecanora invadens grows on non-calcareous or weakly calcareous rock and is mostly 'parasitic' on other lichens (e.g., *Aspicilia*, *Collema*, *Endocarpon*, *Lecanora, Verrucaria*). It is rarely found on man-made substrata. New to Turkey and the Middle East, where it was accompanied by *Aspicilia calcarea*, *Collema tenax*, *Endocarpon* sp., and *Fulgensia fulgens.* Also known from Europe, Asia, and North America (Sliwa 2007).

Specimen examined: **TURKEY. Burdur:** Bucak, surroundings of Karacaören Dam, 37°21′35.14″N 30°50′05.80″E, 300 m, on *Endocarpon* sp., *Collema tenax*, and soil, 25.06.2012, leg. K. Yazici. (KTUB–2336).

Remarks— *Lecanora invadens* resembles *L. semipallida* but has dark brown to slightly black and slightly pruinose apothecia with a yellow-brown epithecium, and a more distinct thallus.

Lecidea promiscua Nyl., Flora 57: 357. 1874.

Thallus thin, indistinctly areolate-rimose and sordid-white; prothallus not developed or indistinct; areoles flat, irregular and indistinct; medulla white, I+ intensely violet. Apothecia sessile, with markedly or strongly constricted base, to 2.5(–4) mm in diam.; disc black, flat to ± convex, emarginate, epruinose or slightly pruinose; margin black, persistent, dull or ± shiny; exciple black, blue-green to brown-black in a thin, peripheral rim, 60–150 µm wide; epithecium dark blue-green to green-brown-black, 12–18 µm thick; hymenium hyaline or faintly green, 46–58 µm tall; paraphyses simple, occasionally branched, lax or contiguous, especially towards the apical ends; hypothecium dark-brown below, lens-shaped, hyaline above; asci clavate 8-spored, 40–50 × 13–17µm; ascospores hyaline, simple, oblong to oblong-ellipsoid, (7–)9–12.5 × (2.5–) 3–4.5 µm. Pycnidia semi-immersed, 90–150 µm in diam.; conidia bacilliform to filiform, straight, (9–)9.5–14 × 1–1.3 µm. C–, K–, KC–, Pd–, UV–.

Lecidea promiscua is a circumpolar, arctic-alpine to boreal-montane lichen, growing on granite boulders close to the ground and on siliceous pebbles in wind-exposed and sunny situations widespread in the Alps. New to Turkey and the Middle East, where it accompanied *Aspicilia desertorum, A. cinerea, Acarospora fuscata, Caloplaca saxicola, Immersaria athroocarpa,* and *Lecanora muralis.* Also known from Europe and Central Asia (Hertel 2006).

Specimen examined: **TURKEY. Van:** Saray-Kapiköy, vicinity of Iran border, 38°29′57″N 44°17′33″E, 2276 m, on siliceous rock, 28.09.2009, leg. A. Aslan. (ATAKKEF–1450).

Remarks— *Lecidea promiscua* resembles *L. promiscens,* which is distinguished by a thick white thallus.

Melaspilea interjecta (Leight.) A.L.Sm., Monogr. Brit. Lich. 2: 228. 1911.

Thallus thin, pale white-green-grey to pale fawn or brown; photobiont cells 5–16 µm in diam., trebouxioid. Apothecia 0.4–1.2 × 0.2–0.3 mm, lirellate, simple or forked, at with a black, slit-like, later widening disc and a tumid margin, later developing additional slits or becoming subgyrose-contorted; true exciple well developed, reddish brown-black, K± olivaceous; epithecium pale to dark brown, K± olivaceous; hymenium 70–85 µm tall, colourless, I+ yellowish, K/I+ blue; hypothecium concolorous with exciple; paraphyses 1.5–2 µm wide, richly branched and anastomosing. Asci 47–53 × 21–26 µm, 8-spored; apical dome with narrow ocular chamber. Ascospores 17–23 × 7–9 µm, 1-septate, sickle-shaped, constricted and easily broken at the septum, colourless but old spores brown and finely warted.

Melaspilea interjecta grows on vertical, smooth surfaces of slate rocks and mica-schist rocks. New to Turkey and Asia. Also known from Europe (Smith et al. 2009).

Specimen examined: **TURKEY. Van:** Çatak, vicinity of waterfall, 38°03′10″N 43°02′59″E, 1689 m, on *Aspicilia calcarea* on calcareous rock, 16.09.2009, leg. A. Aslan. (ATAKKEF–1451).

Remarks— *Melaspilea interjecta* has usually the appearance of an *Opegrapha,* while aged specimens with subgyrose apothecia are likely to be confused with *Polysporina simplex.* The bilocular ascospores are distinctive.

Placynthium garovaglioi (A. Massal.) Malme, Lichenes Suecici Exs.: 734. 1918.

Thallus thick, brown in the centre, with green-brown lobe tips, small, 0.7 – 1.5 cm wide, with at least part of the margin obscurely lobed; thallus lobes very slender; lobes 0.1–0.12 mm wide, 0.4–1 mm long; marginal lobes, when present, are very slender; thallus centre is uneven; thallus with dense, flat to convex, nodular-scurfy squamules 0.08–0.25 mm in diam.; the entire thallus powdery with dense, bluish-white pruina, ± dull grey-brown or ± green-brown when wet; blue-black prothallus occasionally visible at tips and margins of the lobes. Apothecia not seen.

Placynthium garovaglioi grows in patches along fissures on sheltered, vertical to overhanging, dry, and on steeply inclined, sunny calcareous rocks with some water seepage. New to Turkey and Asia, where it accompanied *Aspicilia, Calcarea, Caloplaca lactea*, *Caloplaca variabilis, Candelariella aurella,*

Dermatocarpon miniatum, and *Verrucaria calciseda.* Also known from Europe (Czeika & Czeika 2007).

Specimens examined: **TURKEY. Van:** Bahçesaray, exit of district, 38°06′08″N 42°48′41″E, 1587 m, on calcareous rock, 14.09.2009, leg. A. Aslan, (ATAKKEF–1452). **Iğdır:** Tuzluca, Kula village, roadside, 40°51′4.25″N 43°25′53.48″E, 1175 m, on calcareous rock, 12.07.2010, leg. K. Yazici. (KTUB–2337).

Remarks— *Placynthium garovaglioi* is similar to *P. hungaricum,* but has appressed marginal lobes and central parts with tightly packed squamules instead of appressed, and central parts have branched isidioid lobules as in *P. hungaricum.* In addition, the ascospores in *P. hungaricum* are 1-septate.

Verrucaria bryoctona (Th. Fr.) Orange, Lichenologist 23: 3. 1991.

Thallus superficial, 0.9–1.4 cm in diam, yellow-green, granular-verrucose, composed of goniocyst-like units 15–40 µm in diam., without pigment in cell walls. Photobiont green, cells isodiametric to broadly rectangular, 5.5–10 × 4–7.5 µm. Perithecia spherical to broadly ovoid, 0.12–0.28 mm in diam., black, immersed in the substratum, ostiole pale grey, exciple pigmented throughout; involucrellum absent. Excipulum 20–40 µm thick, of textura angularis, darkly pigmented or slightly paler at base, ± reddish-brown, K+ dark grey-brown. Periphyses up to 25–35 × 2–2.5 µm, branched, sparingly anastomosing at base. Asci clavate, 60–75 × 12–17 µm, fissitunicate. Ascospores narrowly ellipsoid, 1(–3)-septate when mature, 19.5–26.5 × 5–7 µm, apices rounded or ± truncate with a small gelatinous appendage 2–3.5 µm wide and 1 µm long. Pycnidia absent.

Verrucaria bryoctona grows on slightly to strongly basic soil, usually with acrocarpous mosses, sometimes, overgrowing dead moss, in dry grassland. It occurs occasionally in semi-natural and man-made habitats such as dunes, waste ground, spoil heaps, and wall tops. New to Turkey and Asia, where it was accompanied by *Bilimbia sabuletorum, Cladonia pocillum,* and *Collema tenax.* Also known from Europe and North America (Orange 1991).

Specimen examined: **TURKEY. Burdur:** Bucak, surroundings of Karacaören dam, 37°21′35.14″N 30°50′05.80″E, 300 m, on soil and mosses, 25.06.2012, leg. K. Yazici. (KTUB– 2335).

Remarks— *Verrucaria bryoctona* is similar to *V. xyloxena,* which is distinguished by less elongated ascospores, the absence of a small gelatinous appendage, and a brown pigment in the thallus.

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Ophiodothella angustissima comb. nov., a new name for *Acerviclypeatus poriformans* and *O. vaccinii*

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Abstract —A common leaf spot disease on *Vaccinium arboreum* in the southeastern United States has been attributed to *Ophiodothella vaccinii*. The leaves are infected in early spring and during the summer abundant acervular conidia are produced. In addition to *O. vaccinii*, two names have been applied to this fungus in the literature, *Septoria angustissima* and *Acerviclypeatus poriformans*. A study was undertaken to determine which name should be applied to this fungus, resulting in our proposing a new combination. On the basis of priority, *O. angustissima* comb. nov. is the correct name for the taxon.

Key words — anamorph, *Ascomycota*, *Phyllachoraceae*, sparkleberry, taxonomy

Introduction

Vaccinium arboreum Marshall (sparkleberry) is a common understory shrub in the southeastern United States. The leaves of this plant are frequently infected by *Ophiodothella vaccinii*, which causes a leafspot commonly known as "fly speck" disease. In her original description of the species, Boyd (1934) described the anamorph as an acervulus, but without assigning it to a particular genus. Hanlin (1990a) published illustrations of the species and based on the unique morphology of the anamorph, he concluded that it could not be accommodated in any current anamorph. He subsequently assigned it to the new genus *Acerviclypeatus* as *A. poriformans* (Hanlin 1990b). Barr (1990), however, in her review of pyrenomycetous genera, indicated that *Septoria angustissima* (Peck 1911) is an earlier name for *O. vaccinii*. Consequently, a study was undertaken to determine the correct name for this fungus.

Materials & methods

Type specimens of *A. poriformans*, *O. vaccinii* and *S. angustissima* were examined and compared visually. Because of the sparseness of material in the type of *S. angustissima*,

no mounts were made, especially since the original description matches the later descriptions in Boyd (1934) and Hanlin (1990a), which were confirmed by Barr (1990).

Results

After close examination, it is clear that the three examined type specimens *Acerviclypeatus poriformans*, *Ophiodothella vaccinii,* and *Septoria angustissima* represent the same fungus.

The label on the package containing the holotype specimen of *Septoria angustissima* is shown in Plate 1A. Of significance here is the comment, "On *Maclura pomifera* (Osage orange), leaves." The label on the type packet (Plate 1B) contains the following information, "= *Ophiodothella angustissima* (Pk.) n. comb. [in pencil], Herbarium N. Y. State Museum [printed], TYPE *Septoria angustissima* Peck [in ink] Aberdeen, Miss. on lvs. of Osage orange [in ink] (host is *Vaccinium arboreum*) [in pencil], T.C. Frye, 28 Aug. 1906 [in ink]."

Inside the type packet of *S. angustissima* are three dried leaves of the specimen, two intact and the other with a portion missing (Plate 2A). Also in the packet are two additional leaves and several notes:

(1) A handwritten note in ink (Plate 2B) bears the notation, "on leaves of Osage orange growing wild in river bottoms, Aberdeen, Miss. Coll. T.C. Frye. No. 26. Aug. 28, 1906." Above this is a penciled note, "I'm at a loss to understand why Peck called this a *Septoria*. (HDH)."

(2) A typewritten note (Plate 3A) states, "The host is not *Toxylon* (a loose leaf of *Toxylon*, with its acuminate apex enclosed), but seems to match the smaller leaf enclosed which is *Vaccinium arboreum* March. [sic] (*Batodendron arboreum* Nutt.). Do you agree?" Two leaves are included with this note. *Toxylon* is a synonym of *Maclura*, the genus of Osage orange.

(3) A handwritten 8.5 × 8.5 cm note (Plate 3B) says, "I compared these with mulberry leaves. The upper surfaces are similar but the cuticles below are different. The edges are similar. The fungus is immature. Can you collect again about the 1st of May?" On the reverse side of this note (Plate 4A) are the comments:

> "I share your wonder why this was described as a *Septoria*. The housing is not a typical pycnidium. The raphe-like sheaves beside the globose cells remind one of the conidia of some Entylomata. They may not be reproductive organs.
>
> "The units seem to consist of a carbonous clypeus over an acervulus.
>
> "I have retained the leaf I was working on to seek it among the [unclear] of the heaths" over initials [unclear].

4) A handwritten note (Plate 4B) is: "Conidial stage of *Ophiodothella vaccinii* Boyd M. E. Barr Bigelow May 1963".

NEW YORK STATE MUSEUM TYPE COLLECTION
Septoria angustissima Peck
Holotype
Leg. T.C.Frye (comm. F.D. Kern)
Aberdeen MS USA
On Maclura pomifera (Osage orange), leaves
Type database entry#: 0204

A

= Ophiodothella angustissima (Pk.) n. comb.
HERBARIUM N. Y. STATE MUSEUM TYPE
Septoria angustissima Peck
Aberdeen, Miss. on lvs. of Osage orange.
(Host is Vaccinium arboreum)
T. C. Frye, 28 Aug. 1906.

B

PLATE 1. *Septoria angustissima* (holotype collection). A: Label. B: Front of packet.

Discussion

Although the leaves of *Maclura* and *Vaccinium* resemble one another superficially in being broadly elliptical and dark green in color, the leaves of *Maclura* are larger (7.5–13 cm long × 5–7.5 cm wide) and end in an acuminate tip, whereas the leaves of *Vaccinium* are smaller (3–7 cm long × 2–4 cm wide), with an obtuse apex. The plants also differ greatly in size; *Maclura* is a large tree,

PLATE 2. *Septoria angustissima*.
A: Type specimen. B: Note with collection information for the holotype.

whereas *Vaccinium* is a shrub or occasionally a small tree. The leaves of the type specimen are clearly those of *V. arboreum*.

The structure of the conidioma, which Peck termed a perithecium, is an acervulus, whereas in *Septoria* the conidia are formed in a well-defined

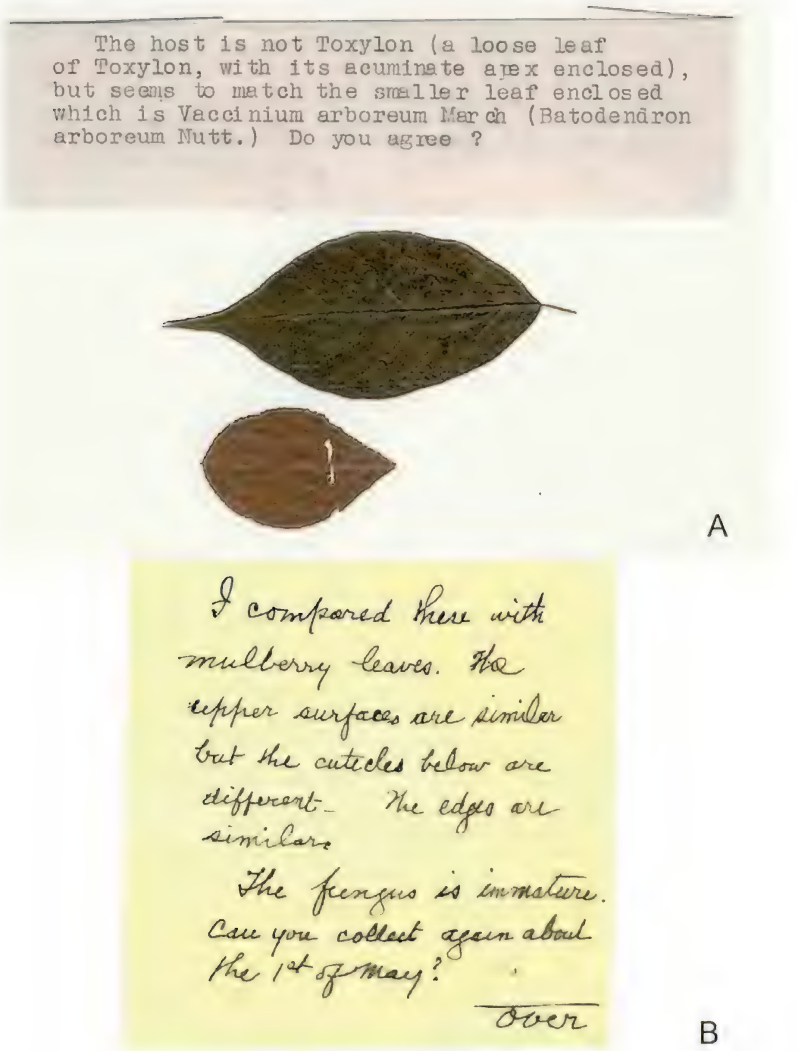
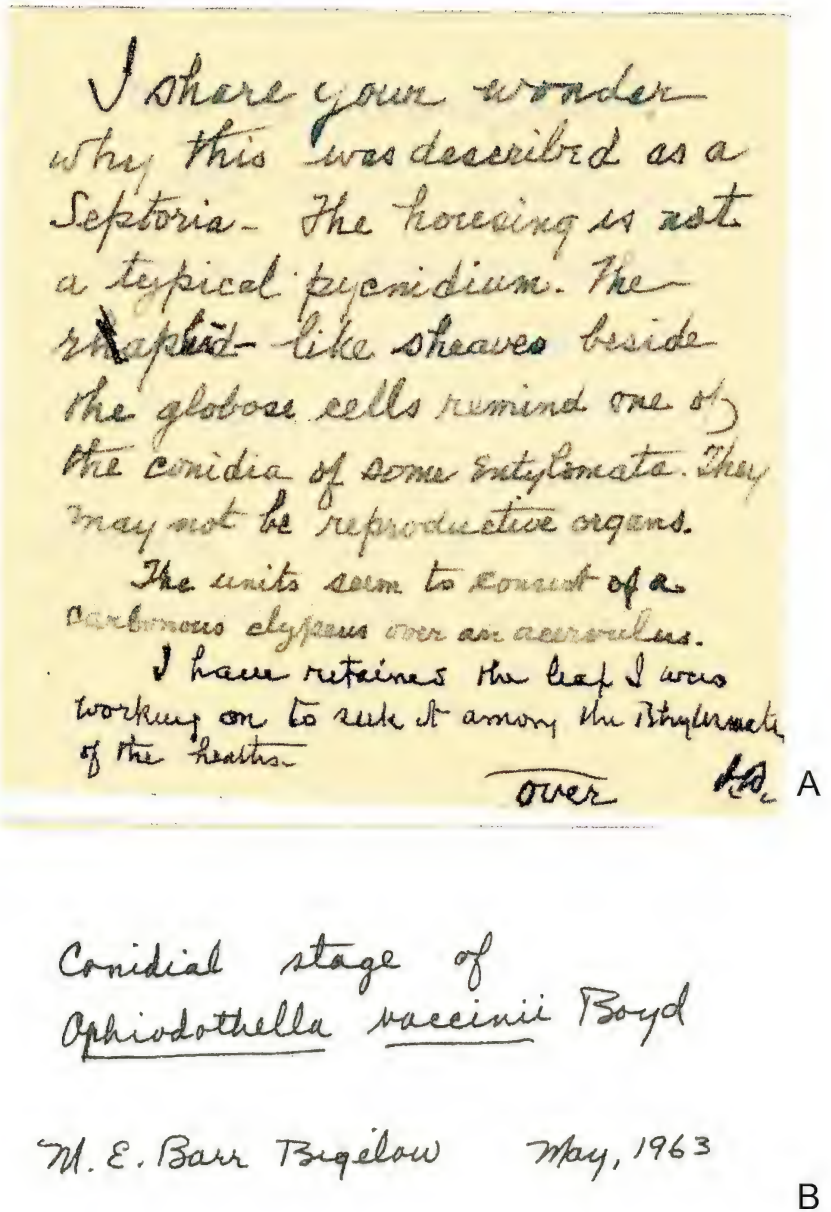

The host is not Toxylon (a loose leaf of Toxylon, with its acuminate apex enclosed), but seems to match the smaller leaf enclosed which is Vaccinium arboreum March (Batodendron arboreum Nutt.) Do you agree ?
A
I compared these with mulberry leaves. The upper surfaces are similar but the cuticles below are different. The edges are similar.
The fungus is immature. Can you collect again about the 1st of May?
over
B

PLATE 3. A: Note comparing a leaf of *Maclura* (=*Toxylon*) *pomifera* (upper) with one of *Vaccinium arboreum* (lower). B: Note comparing mulberry leaves (not shown) with the leaves in 3A.

pycnidium (Sutton 1980). Although the conidia in both *O. vaccinii* and *Septoria* are hyaline and filiform, *Septoria* conidia are usually septate, whereas the conidia of *O. vaccinii* are aseptate. Also, conidia in *Septoria* tend to be larger then those in *Ophiodothella*; Sprague (1944) showed that conidia of *Septoria* from grasses


I share your wonder why this was described as a Septoria. The housing is not a typical pycnidium. The rhaphid-like sheaves beside the globose cells remind one of the conidia of some Entylomata. They may not be reproductive organs.

The units seem to consist of a carbonous clypeus over an acervulus.

I have retained the leaf I was working on to seek it among the Rhytismata of the heaths.

over

A

Conidial stage of
Ophiodothella vaccinii Boyd

M. E. Barr Bigelow May, 1963

B

Plate 4. *Septoria angustissima* (holotype collection). A: Note with comments regarding identification of host leaves. B: Annotation by M. E. Barr Bigelow.

varied in size from 22–98 µm long × 1.4–2.6 µm wide, compared with 18–24 × 1 µm in *Ophiodothella*. This is implied by Peck (1911) in his description of the species as, "Remarkable for its very narrow spores." In addition, the *Vaccinium* anamorph has an unusual method of pore formation, as shown in a study of ontogeny of the conidioma (Hanlin 2003). This served as the basis for assigning it to the genus *Acerviclypeatus* (Hanlin 1990b).

The penciled annotation at the top of the type packet, " = *Ophiodothella angustissima* (Pk.) n. comb.," is interesting, as it indicates that the connection of the specimen with *Ophiodothella* was recognized early. When and by whom this annotation was made is unknown, but the name appears to have not been published. In Index Fungorum (CABI 2012), *S. angustissima* is listed as "anamorphic *Mycosphaerella*" but this refers only to the generic anamorph/teleomorph connection between *Septoria* and *Mycosphaerella*. *Ophiodothella vaccinii* also has been mistakenly identified as *Rhytisma vaccinii* (Schwein) Fr. due to the similarity in appearance of the acervuli with the apothecia of certain species of *Rhytisma*, such as *R. punctatum* (Pers.) Fr. *Rhytisma vaccinii* was described on *V. frondosum* from North Carolina.

Prior to the description of the genus *Acerviclypeatus* a search of the literature was made to look for similar fungi reported on *V. arboreum*. Alfieri et al. (1984) reported *Septoria albopunctata* Cooke on *V. arboreum* from Florida, and Farr et al. (1989) list the same species from South Carolina and Texas. The report of *S. angustissima* was overlooked because of the misidentification of the host.

Based on the above results, a new combination is proposed for the fungus causing the leaf spot disease on *Vaccinium arboreum*.

Ophiodothella angustissima (Peck) Hanlin & M.C. González, **comb. nov.** Pl 1–4

MycoBank MB 803907

≡ *Septoria angustissima* Peck, Bull. N. Y. St. Mus. 150: 62. 1911.

= *Ophiodothella vaccinii* Boyd, Mycologia 26: 465. 1934.

= *Acerviclypeatus poriformans* Hanlin, Mycotaxon 37: 381. 1990.

specimens examined, **UNITED STATES. Georgia: Clarke Co.,** Athens, on leaves of *Vaccinium arboreum,* April 1933, Sophie Boyd (GAM 7026 [=M 7091], holotype of *Ophiodothella vaccinii*). **Madison Co.,** on leaves of *Vaccinium arboreum,* 10 September 1989, RT Hanlin (903) (GAM 12745, holotype of *Acerviclypeatus poriformans*); **Mississippi: Monroe Co.,** Aberdeen, on leaves of "Osage orange" [actually *V. arboreum*], 28 August 1906, TC Frye (NYS 0204, holotype of *Septoria angustissima*).

On the basis of the priority of its epithet, *O. angustissima* is the correct name for the taxon.

Acknowledgments

We thank the curators of NYS for loan of the type of *S. angustissima*. The senior author is grateful to the late Margaret Barr for calling his attention to her remarks regarding this fungus. We thank Sabine Huhndorf and Amy Rossman for their helpful reviews of the manuscript.

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The genus *Wrightoporia* in Korea

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Abstract —The genus *Wrightoporia (Hericiales, Basidiomycota)* and two species, *W. japonica* and an undetermined *Wrightoporia* sp. are reported as new to Korea. *Wrightoporia luteola* is treated as a synonym of *W. japonica*. Illustrated descriptions and a key to Korean *Wrightoporia* species are provided.

Key words — ITS, nuc-LSU, phylogeny, polypore, taxonomy

Introduction

Wrightoporia Pouzar is a genus of wood-rotting fungi. The current generic concept is broad and the genus includes species with: (1) resupinate to pileate basidiocarps; (2) poroid hymenophore; (3) smooth to ornamented and amyloid basidiospores; and (4) diverse hyphal characters such as monomitic or di–trimitic hyphal system, generative hyphae with or without clamp connections, and skeletal hyphae with or without a dextrinoid reaction. Some species also have gloeoplerous hyphae (Hattori 2008). A total of 38 *Wrightoporia* species have been described so far according to Index Fungorum databases (http://www.indexfungorum.org/Names/Names.asp), and they have been found in both tropical to temperate areas. Of the 18 species reported from Asia, some have been described from Asia (Hattori 2008), but none have been recorded in Korea.

During studies of indigenous fungi, especially polypores, we found three *Wrightoporia* specimens. Our examinations and sequence analysis established that they represented *W. japonica* and an undetermined *Wrightoporia* sp. We also determined that *W. luteola* is a taxonomic synonym of *W. japonica*.

Materials & methods

Collection and morphological examination

Basidiocarps were collected from Mt. Guryong and Mt. Dobong in Seoul, Korea, and Mt. Songni in Chungcheongbuk-do, Korea, in 2011. They were dried completely overnight using an air drier at 40°C. Slide preparations were made from dried specimens mounted in 5% (w/v) KOH, 1% (w/v) phloxine and Melzer's reagent (Largent et al. 1977) using an Olympus BX51 light microscope (Olympus, Tokyo, Japan). The abbreviations used in the text are as follows: IKI– = inamyloid, L = mean spore length, W = mean spore width. Munsell soil color charts (Munsell Color 2000) was used as the color standard. The voucher specimens were deposited at the National Institute of Biological Resources, Incheon, South Korea (KB) with the acronym KUC (Korea University Culture Collections, Korea University, Seoul, South Korea).

Phylogenetic analysis

The genomic DNAs were extracted according to Jang et al. (2012a). PCR amplification reactions were performed with the primers LR0R/LR3 or LR0R/LR5 for nuclear large subunit ribosomal DNA (nuc-LSU) region (Vilgalys & Hester 1990) and ITS1F/ITS4 for internal transcribed spacer (ITS) region (Gardes & Bruns 1993, White et al. 1990) according to Jang et al. (2012a). The DNA sequencing was performed at the DNA sequencing center (Macrogen, Korea). The obtained sequences were deposited in GenBank. They were aligned with the reference sequences selected by BLASTn search of GenBank using MAFFT 6.885, L-INS-i alignment method (Katoh & Toh 2008). Each dataset was manually edited using MacClade 4.08 (Maddison & Maddison 2005). The aligned nuc-LSU dataset of 20 taxa comprised 911 characters, and the aligned ITS dataset of 19 taxa comprised 656 characters. Gaps were treated as missing. GTR + I + G model was applied for both of the datasets via MrModeltest 2.3 under the AIC (Nylander 2004). Bayesian analysis of each dataset was performed with MrBayes 3.2.1 (Ronquist et al. 2012). A 50% majority-rule consensus tree for each dataset was constructed according to Jang et al. (2012b). Two runs with 1,000,000 generations were performed and every 100th generation was sampled. Among them, the last 75% sampled trees were used. The tree was viewed using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Taxonomy

Wrightoporia japonica Núñez & Ryvarden, Fungal Diversity 3: 119. 1999. FIG. 1

= *Wrightoporia luteola* B.K. Cui & Y.C. Dai, Nova Hedwigia 83: 160. 2006.

BASIDIOCARPS annual, resupinate to effused-reflexed, adnate, ca. 13.5 cm or more in longest dimension, ca. 5.5 cm in widest dimension in Korean specimens. Sterile margin narrow, obtuse, felty-corky, very pale brown (10YR8/4), less than 1 mm wide. Pileus elongated, up to 6 mm wide in Korean specimens; pileus

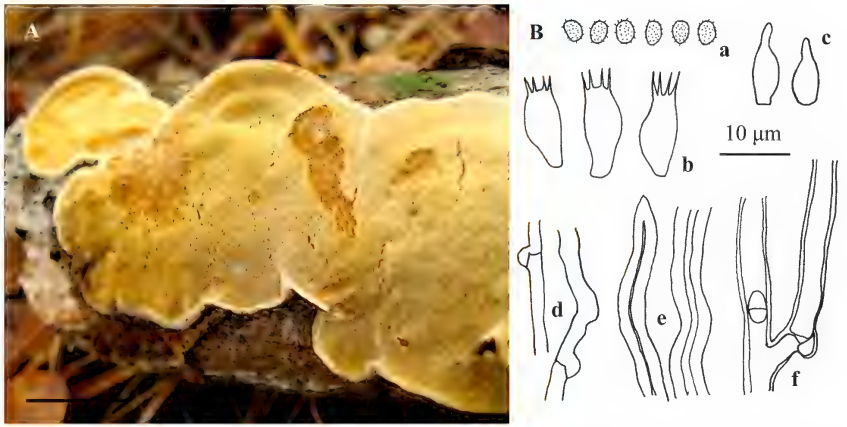

Fig. 1. *Wrightoporia japonica*. A. Basidiocarp (KUC20111019A-29). Scale bar = 1 cm. B. Microscopic features (a–e, KUC20110908-13; f, KUC20111019A-29). a, basidiospores; b, basidia; c, cystidioles; d, generative hyphae from trama; e, skeletal hyphae from trama; f, generative hyphae from context.

surface glabrous, dull, brownish yellow (10YR6/6), often darker near the base; pileus margin thin, entire, somewhat darker than the sterile margin, less than 1 mm wide. Pores round to angular, 7–8/mm in Korean specimens; dissepiments thin and entire; pore surface very pale brown (10YR8/4) to brownish yellow (10YR6/8) in dried condition, slightly shiny when turned in incident light. Subiculum felty-corky, concolorous to the pore surface, up to 1 mm thick. Tubes felty-corky, pale yellow, up to 1 mm deep.

Hyphal system dimitic; generative hyphae with clamp connections and skeletal hyphae.

Subiculum generative hyphae with clamp connections, hyaline, thin to slightly thick-walled, 1.5–4.5 μm in diameter; skeletal hyphae hyaline to yellowish, thick-walled, occasionally branched, straight to sinuous, 3–6 μm in diameter, most with a narrow lumen, some hyphae covered with crystals, interwoven, IKI–.

Tubes generative hyphae with clamp connections, hyaline, thin to slightly thick-walled, 1.5–3 μm in diameter; skeletal hyphae abundant, hyaline to yellowish, thick-walled, unbranched, straight to sinuous, 2.5–5 μm in diameter, mostly with a narrow lumen, some hyphae covered with crystals, interwoven, partly dextrinoid especially in the dissepiments; cystidia none, fusoid cystidioles present in one specimen (KUC20111019A-29); basidia oblong ellipsoid to clavate, 4 sterigmate, 9–12.5 × 4–5.5 μm.

Basidiospores broadly ellipsoid to subglobose, hyaline, thin-walled, with finely asperulate ornamentations, amyloid, 2.6–3.5 × 1.6–2.6 μm, L = 3.25 μm, W = 2.28 μm.

Type of rot— white rot.

Specimens examined: **KOREA**, **Seoul**, Mt. Guryong, 37°28′43″N 127°04′04″E, on fallen wood branch, 08 September 2011, Yeongseon Jang (KB, KUC20110908-13; GenBank KC166692). **Chungcheongbuk-do**, Mt. Songni, 36°38′07″N 127°25′49″E, on fallen wood branch, 19 October 2011, Yeongseon Jang, (KB, KUC20111019A-29; GenBank KC166693). **JAPAN**, **Okinawa Pref.**, Iriomote Isl., riverside of Shiira River, 19 June 1994, M. Núñez (isotype of *W. japonica*, TFM F-20724). **CHINA. Anhui Prov.**, Huangshan County, Yellow Mts., 13 October 2004, B.K. Cui (isotype of *W. luteola*, TFM, ex Dai 6199); **Fujian Prov.**, Wuyishan, the Virgin Forest Park, Longfenggu Scenicspot, 19 October 2005, Y.C. Dai (TFM, ex Dai 7221).

Distribution — China, Japan and Korea.

Remarks — *Wrightoporia japonica* was first described by Núñez & Ryvarden (1999), and Hattori (2008) gave a more detailed description based on ten specimens including the isotype. An examination of the *W. luteola* type showed it to be a resupinate form of *W. japonica*, which is morphologically similar to *W. gillesii* A. David & Rajchenb. (Hattori 2008). Further study is desirable to reveal whether they represent two distinct species.

***Wrightoporia* sp.** Fig. 2

Basidiocarps annual, resupinate, adnate, and confluent. Pores angular, 5–6/mm, dissepiments thin and entire, partly eroded; pore surface very pale brown (10YR8/4). Marginal sterile zone indistinct to membranous, very pale brown (10YR8/2), up to 2 mm wide. Subiculum very thin, less than 0.5 mm. Tubes concolorous to the pore surface, up to 1 mm deep.

Hyphal system dimitic; generative hyphae with clamp connections and skeletal hyphae.

Subiculum generative hyphae with clamp connections, hyaline, thin to slightly thick-walled, 1.5–5 μm in diameter; skeletal hyphae hyaline to yellowish, thick-walled, occasionally branched, straight to sinuous, 2.5–6 μm in diameter, mostly with a narrow lumen, interwoven, IKI–.

Tubes generative hyphae with clamp connections, hyaline, thin to slightly thick-walled, 1.5–3.5 μm in diameter; skeletal hyphae abundant, hyaline to yellowish, thick-walled, unbranched, straight to sinuous, 3–6 μm in diameter, mostly with a narrow lumen, interwoven, partly dextrinoid especially in the dissepiments; cystidia none, fusoid cystidioles scattered; basidia clavate, 4 sterigmate, 10.5–12.5 × 3.5–5 μm.

Basidiospores broadly ellipsoid to subglobose, hyaline, thick-walled, finely asperulate, amyloid, 3.2–4 × 2.4–3.2 μm, L = 3.62 μm, W = 2.85 μm.

Type of rot — white rot.

Specimen examined: **KOREA**, **Seoul**, Mt. Dobong, on fallen wood branch, 22 September 2011, Yeongseon Jang (KB, KUC20110922-37; GenBank KC166694).

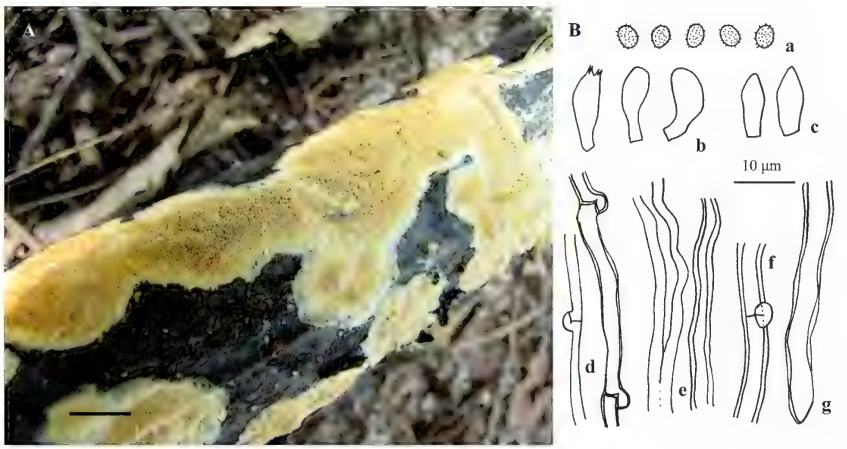

FIG. 2. *Wrightoporia* sp. (KUC20110922-37). A. Basidiocarp. Scale bar = 1 cm. B. Microscopic features. a, basidiospores; b, basidia; c, cystidioles; d, generative hyphae from trama; e, skeletal hyphae from trama; f, generative hyphae from context; g, skeletal hyphae from context.

REMARKS — For the time being, we refer this to *Wrightoporia* sp. because the species outline is ambiguous with only a single specimen available. The specimen is similar to *W. japonica* but is distinguished by the purely resupinate basidiocarps with a very thin subiculum, partly eroded dissepiments, slightly larger spores (3.2–4 μm long), and parallel hyphae in the trama.

Phylogeny

Bayesian analyses of nuc-LSU and ITS regions were performed for the better understanding of phylogenetic relationships with other *Wrightoporia* and allied species available in GenBank. As shown in Larsson & Larsson (2003), *Wrightoporia* species are polyphyletic in both trees (FIG. 3; LSU tree not shown). Our specimens of *W. japonica* (KUC20010908-13 and KUC20111019A-29) have the same nuc-LSU and ITS sequences and form monophyletic clades in both trees. The ITS analysis (FIG. 3) clusters *W. luteola* (Dai 7221 from China) with *W. japonica* with high posterior probability value (1.0 p.p.), separated in the aligned ITS dataset by only two base-pair differences and one gap. This result supports our morphological observation that *W. luteola* is identical with *W. japonica*. *Wrightoporia* sp. KUC20110922-37 was monophyletic with *W. lenta* (Overh. & J. Lowe) Pouzar KN150311 from Jamaica in both trees, but this was only weakly supported (nuc-LSU, 0.54 p.p.; ITS, 0.65 p.p.). *Wrightoporia* sp. KUC20110922-37 is morphologically similar to *W. japonica*, and the *Wrightoporia* sp.–*W. lenta* clade clustered with *W. japonica* in the nuc-LSU tree (not shown). The ITS tree does not resolve the relationships among

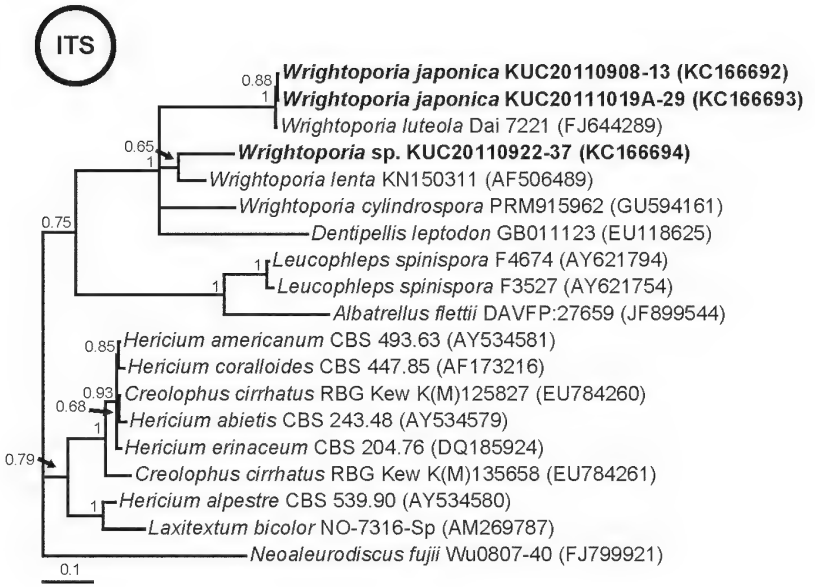

FIG. 3. ITS region 50% majority-rule consensus tree of *Wrightoporia* spp. and allied species. For the construction of consensus tree, 15,000 trees resulting from Bayesian analysis were used. Posterior probabilities ≥0.50 are shown above branches. GenBank Accession numbers of the sequences are shown in parentheses.

Wrightoporia sp.–*W. lenta* clade, *W. japonica*, *W. cylindrospora* Ryvarden, and *Dentipellis leptodon* (Mont.) Maas Geest. (FIG. 3). Further study with more *Wrightoporia* samples is required to determine the species relationships and the identity of our undetermined *Wrightoporia* sp.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390) and was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea. TH wishes to express his thanks to Prof. Y.C. Dai (Beijing Forestry University) for the deposit of *W. luteola* specimens in TFM. We are grateful to Dr. Leif Ryvarden and Dr. Ota Yuko for their valuable suggestions on the manuscript.

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Three new cercosporoid fungi from the Brazilian Atlantic Forest

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Abstract — Three new cercosporoid hyphomycetes were found associated with leaf spots on three host families in a stretch of Atlantic Forest in Viçosa, Minas Gerais, Brazil: *Pseudocercospora aurelianae* sp. nov. on *Aureliana velutina* (*Solanaceae*), *P. vicosae* sp. nov. on *Eupatorium* sp. (*Asteraceae*), and *Passalora rolliniae* sp. nov. on *Rollinia sylvatica* (*Annonaceae*). These three species are fully described, illustrated, discussed, and compared with allied species in the present work.

Key words — *Capnodiales*, *Dothideomycetes*, *Mycosphaerellaceae*, taxonomy, tropical fungi.

Introduction

Brazil is considered one of twelve countries with so-called mega-diversity. Covering the major biomes, the Atlantic Forest is regarded as one of the greatest repositories of biodiversity on the planet with ca. 20,000 plant species, of which 8000 are endemic (Myers et al. 2000). For example, in a single region in southern Bahia state, 450 angiosperms were reported per hectare (Varjabedian 2010). Because the Atlantic Forest has been reduced to 7.5 % of its original extent (Myers et al. 2000), the study of mycobiota in this endangered biome has priority.

The lack of knowledge of the mycobiota associated with Atlantic Forest plants can best be improved by comprehensive examination of certain fungal groups (Pinho et al. 2012). We decided to study cercosporoid hyphomycetes, as the knowledge of the diversity of this fungal group in tropical environments is still sparse. In addition, cercosporoids are among the largest groups of anamorphic fungi and are commonly associated with leaf spot diseases on almost all families of flowering plants worldwide (Crous & Braun 2003). First studies of cercosporoids in Brazil were made by Viégas and Batista (Viégas 1945; Silva & Minter 1995).

During the last decades, several papers have dealt with cercosporoid fungi from Brazil (Crous et al. 1997, 1999, 2011; Crous & Câmara 1998; Barreto et al. 1999). The majority of species have been reported in other biomes, mainly cerrado (Dianese & Câmara 1994; Medeiros & Dianese 1994; Inácio et al. 1996; Dianese et al. 1998, 1999; Braun et al. 1999; Furlaneto & Dianese 1999; Inácio & Dianese 1998, 1999; Braun & Freire 2002, 2004, 2006; Dornelo-Silva & Dianese 2003; Dornelo-Silva et al. 2007; Hernández-Gutiérrez & Dianese 2008, 2009; Freire & Braun 2009). Sporadic works on cercosporoid fungi in Atlantic Forest fragments revealed some new, undescribed species, such as the first reports of cercosporoid fungi on hosts of the family *Siparunaceae* and a revision of cercosporoids on *Piperaceae* (Pereira et al. 2003, 2006, 2007; Vieira & Barreto 2004; Pereira & Barreto 2005, 2006; Soares & Barreto 2005; Rocha et al. 2007, 2008, 2010; Silva & Pereira 2007, 2008, 2010, 2011; Alves et al. 2008; Macedo & Barreto 2008; Almeida et al. 2010; Alves & Barreto 2010; Machado et al. 2012; Silva et al. 2012). In this paper, we introduce three new species, one on *Solanaceae*, one on *Annonaceae*, and one on *Asteraceae*.

Material & methods

Small samples of the fungi were removed from fresh leaf spots and mounted in lactophenol. Observations, measurements and drawings were prepared using a Carl Zeiss Standard W light microscope (Göttingen, Germany). Representative specimens of the fungi were deposited in the herbarium of the Universidade Federal de Viçosa (Herbarium VIC).

Taxonomy

Pseudocercospora aurelianae Firmino & O.L. Pereira, **sp. nov.** PLATE 1

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Differs from *Pseudocercospora carolinensis* and *P. marcelliana* by its light brown conidia, and from *P. modesta* by its shorter conidiophores and narrower conidia.

TYPE: Brazil, Minas Gerais, Viçosa, Estação Experimental Mata do Paraíso, on leaves of *Aureliana velutina* Sendtn. (*Solanaceae*), 25 May. 2009, A. L. Firmino (VIC 31847, **holotype**).

ETYMOLOGY: referring to the host genus *Aureliana*.

Leaf spots epiphyllous, circular or irregular, 4–15 mm diam., necrotic, light brown, with distinct dark brownish margin. Colonies epiphyllous, pale brown. Stromata well developed, intraepidermal, 30–37.5 × 37.5–45 µm, brown. Conidiophores in dense fascicles, arising from stromata, straight, cylindrical to slightly attenuated towards a truncate tip, aseptate, rarely 1-septate, not geniculate, not branched, 8–20.5 × 2.5–4 µm, pale brown, uniform in color, thin-walled, smooth. Conidiophores reduced to conidiogenous cells (one-celled) or conidiogenous cells integrated, terminal, straight to slightly curved,

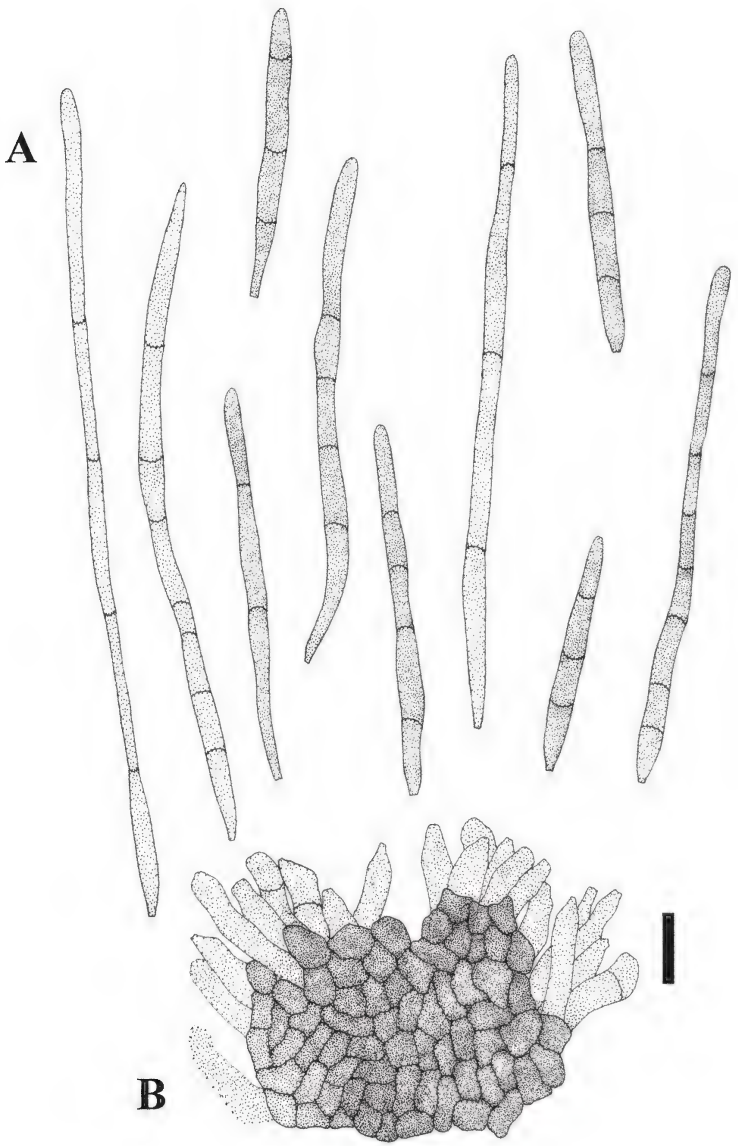

Plate 1. *Pseudocercospora aurelianae*.
A: Conidia. B: Stomata with conidiophores. Bar = 10 µm.

pale brown. Conidiogenous loci inconspicuous or only visible as truncate tip or lateral shoulder in geniculate conidiogenous cells after sympodial proliferation, but always unthickened and not darkened. Conidia solitary, straight to slightly curved, obclavate-subcylindrical to fusiform, 27–100.5 × 2.5–4 µm, 2–7-septate, light brown, smooth, apex obtuse to subacute, obconically truncate at the base, with hilum unthickened and not darkened.

Comments — Twenty-one *Pseudocercospora* species are known to associate with the *Solanaceae* (Chupp 1954; Crous & Braun 2003). The only species reported from Brazil are *Pseudocercospora atromarginalis* on *Capsicum annuum*, *P. daturina* on *Brugmansia suaveolens*, *P. solani-asperi* on *Solanum asperum*, and *P. venezuelae* on *S. argenteum* and *S. gilo* (Crous & Braun 2003). The new species is the first cercosporoid fungus reported on *Aureliana*.

Pseudocercospora aurelianae is morphologically close to *P. carolinensis* (Tharp) U. Braun & Crous and *P. marcelliana* (Chupp) U. Braun & Crous in the lack of superficial mycelium and the presence of well-developed stromata with short conidiophores forming sporodochial conidiomata, but *P. carolinensis* and *P. marcelliana* differ in their hyaline to subhyaline conidia (Chupp 1954). Like *P. aurelianae*, *P. modesta* (Syd.) Deighton has large stromata, but differs by its longer conidiophores (15–75 µm) and much broader conidia (4–6 µm; Chupp 1954).

Pseudocercospora vicosae Firmino & O.L. Pereira, **sp. nov.** Plate 2

MycoBank 800142

Differs from *Pseudocercospora eupatorii* by its shorter conidia.

Type: Brazil, Minas Gerais, Viçosa, Estação Experimental Mata do Paraíso, on leaves of *Eupatorium* sp. (*Asteraceae*), 4 May. 2009, A. L. Firmino (VIC 31848, **holotype**).

Etymology: referring to the type locality.

Leaf spots amphigenous, circular or irregular, 2–11 mm diam., never coalescent, dark brown. Colonies mainly epiphyllous, light brown. Stromata well-developed, intraepidermal, 47.5–70 × 55–97.5 µm diam., brown. Conidiophores loosely fasciculate, arising from stromata, unbranched, flexuous to curved, cylindrical, 18.5–56 × 3–4 µm, 1–2-septate, light brown, paler at the apex, thin-walled, smooth. Conidiogenous cells integrated, terminal, flexuous to curved, pale. Conidiogenous loci inconspicuous or only visible as truncate tip or subdenticulate lateral shoulder formed after sympodial proliferation, but always unthickened and not darkened. Conidia solitary, straight to slightly curved, obclavate to fusiforme, 20–91 × 2.5–4 µm, 1–8-septate, light brown, thin-walled, smooth, apex obtuse to subacute, obconically truncate at the base, hilum unthickened, not darkened.

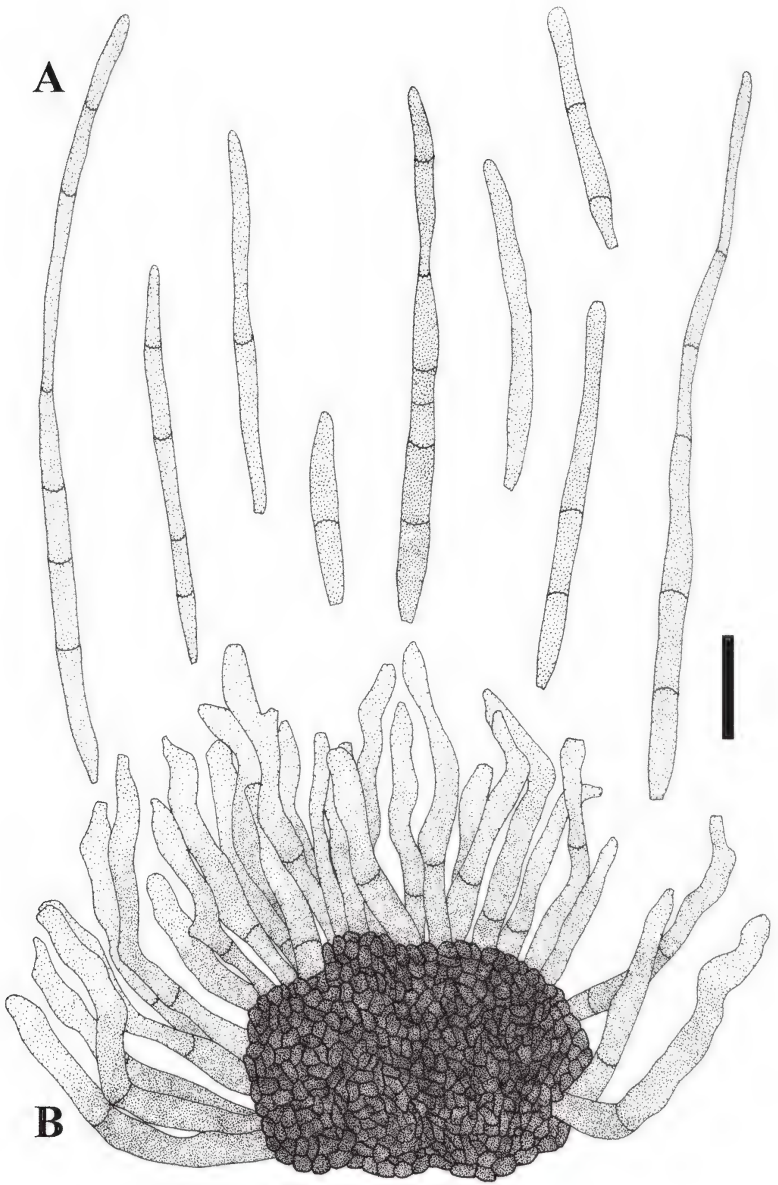

Plate 2. *Pseudocercospora vicosae*.
A: Conidia. B: Stomata with conidiophores. Bar = 10 µm.

Comments — Forty-two *Pseudocercospora* species are known to associate with the *Asteraceae* (Chupp 1954; Braun & Castañeda 1991; Crous & Braun 2003). The only two other species reported from Brazil are *P. elephantopodicola* on *Elephantopus* sp. (Braun & Freire 2006) and *P. plunkettii* on *Mikania micrantha* (Barreto & Evans 1995). Among the forty-two species of *Pseudocercospora* described in association with plants of this family, only *P. aciculina* (Chupp) U. Braun & Crous, *P. ageratoides* (Ellis & Everh.) Y.L. Guo, *P. eupatorii* (Peck) U. Braun & Castañeda and *P. eupatorii-formosani* U. Braun & Bagyan. are associated with *Eupatorium* spp. (Crous & Braun 2003).

Pseudocercospora vicosae is morphologically close to the North American *P. eupatorii* in the lack of superficial mycelium and the presence of well-developed stromata, but *P. eupatorii* differs in its shorter, rarely septate conidiophores (10–35 µm), and longer conidia (50–190 µm; Chupp 1954).

Passalora rolliniae Firmino & O.L. Pereira, **sp. nov.** Plate 3

MycoBank 800144

Differs from *Passalora miliusae* by its much longer and more septate conidiophores, its solitary or catenate light brown conidia, and its annonaceous host plant.

Holotype: Brazil, Minas Gerais, Viçosa, Estação Experimental Mata do Paraíso, on leaves of *Rollinia sylvatica* (A. St.-Hil.) Mart. (*Annonaceae*), 21 January. 2009, A. L. Firmino (VIC 31846, **holotype**).

Etymology: referring to the host genus *Rollinia*.

Leaf spots hypophyllous, circular or irregular, 1–9 mm diam., brown. Colonies mainly hypophyllous. Stroma absent, Conidiophores simple or mostly branched, straight to somewhat curved, 107–361 × 2.5–4.5 µm, 3–7-septate, solitary, erect olivaceous, smooth, thin-walled,. Conidiogenous cells integrated, terminal or intercalate, monoblastic or polyblastic (1–2 loci), olivaceous. Conidiogenous loci conspicuous, somewhat thickened and slightly darkened. Conidia solitary or catenate, straight to flexuous, cylindrical to clavate-obclavate, 6–37 × 2–4.5 µm, 0–3-septate, without constrictions at the septa, obconically attenuated at the base with a thickened and darkened hilum, apex rounded or attenuated, without hilum in solitary conidia or with a single or occasionally two hila in catenate conidia, light brown, thin-walled, smooth.

Comments — Six *Passalora* species are known to associate with the *Annonaceae* (Chupp 1954; Crous & Braun 2003). The only other species reported from Brazil are *Passalora annonigena* on *Annona* spp. and *P. xylopiae* on *A. dioica* and *Xylopia aromatica* (Braun & Freire 2002; Crous & Braun 2003). *Passalora rolliniae* is the first cercosporoid hyphomycete on *Rollinia*.

Passalora rolliniae morphologically resembles *Passalora miliusae* U. Braun & Crous (≡ *Mycovellosiella indica* P. Kumar & Kamal), which differs by its host plant (*Miliusa*, family *Rubiaceae*), its growth habit on the leaf hairs, its much

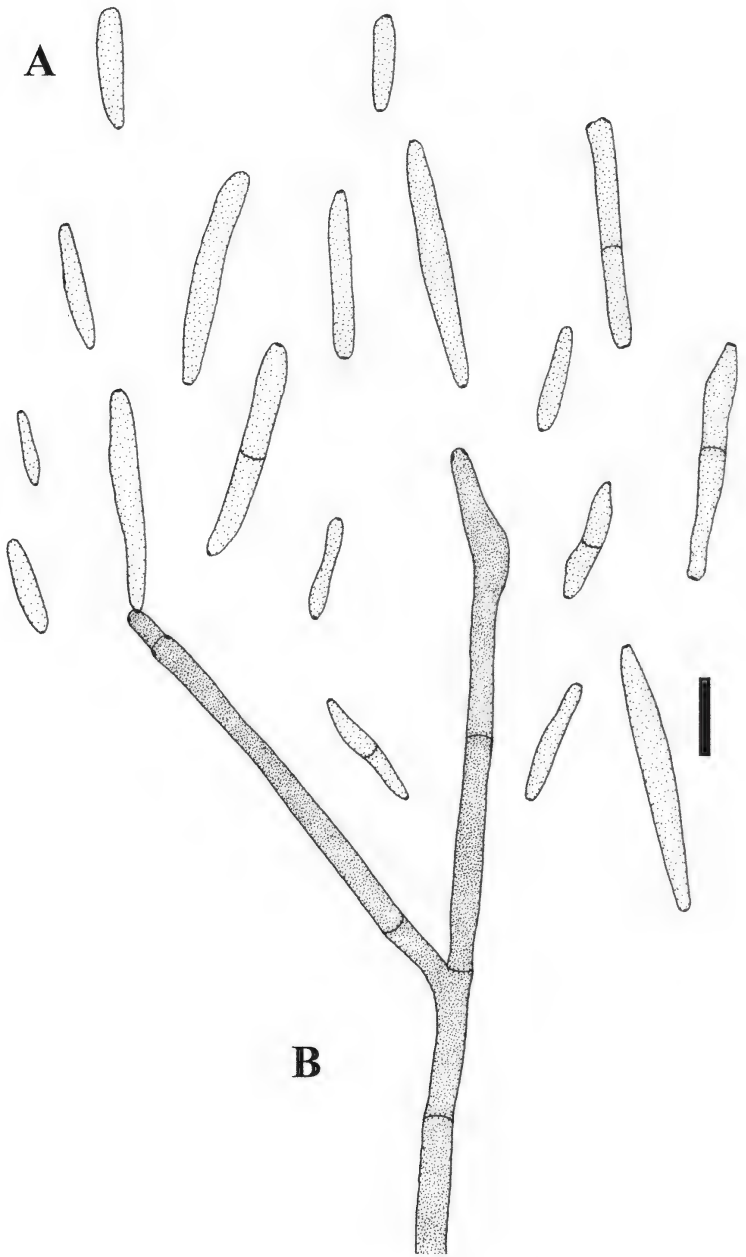

Plate 3. *Passalora rolliniae*.
A: Conidia. B: Conidiophores. Bar = 10 μm.

shorter and less septate conidiophores, and its larger, mostly solitary, subhyaline conidia [14.5–50 × <5.5 µm] (Kumar & Kamal 1982; Crous & Braun 2003).

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A new species of *Entocybe* (*Entolomataceae, Agaricomycetes*) from Québec, Canada

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Abstract — *Entocybe melleogrisea* sp. nov. is described from a subboreal forest type in Québec, Canada. The stature of this species is robust and tricholomatoid and thus is very different from the mostly collybioid species placed in the genus. The distinctive basidiospore morphology, basidiospore histochemistry, and prolific, obvious clamp connections on all hyphae of the basidiomata place this species in *Entocybe*. Preliminary data on ITS sequences support this placement.

Key words — *Agaricales*, mushrooms, North America, key to species

Introduction

A fair number of publications are available on systematics of the *Entolomataceae* Kotl. & Pouzar in North America (Atkinson 1902, 1909; Baroni 1981; Baroni & Petersen 1987; Hesler 1967; Largent 1977, 1994; numerous publications by Peck from 1872 to 1908 [see Murrill 1917 and Noordeloos 2008]; Mazzer 1976; Murrill 1917), but as yet there is no comprehensive monograph of this large and morphologically diverse group of agarics with attached lamellae and flesh-pink angular spores. Although the genus *Entoloma* (Fr.) P. Kumm. s.l. is much better known in Europe (Noordeloos 1992, 2004), even there new species are still being discovered (Noordeloos & Hausknecht 2000; Noordeloos & Morozova 2010). The *Entolomataceae* in North America is a seriously understudied mushroom family. With a minimum of at least 500 species already described from North America (mostly summarized in Hesler 1967 and Largent 1994), it is not surprising that newly discovered taxa are still being found. This report documents one of these distinctive new species.

Baroni et al. (2011) erected the genus *Entocybe* T.J. Baroni et al. in the *Entolomataceae* to recognize a group of species with several unique basidiospore features and a distinctive DNA signature. The basidiospores of *Entocybe* taxa are reminiscent of basidiospores in the genus *Rhodocybe*, i.e., they are subglobose (isodiametric), 6–12 angled in polar view, often obscurely angled in profile view, and the basidiospore surface is adorned with scattered pustules with broken or partial ridging. In addition, all taxa produce obvious clamp connections on the hyphae in all tissues. Taxa with this suite of characters are now confirmed to belong to a basal clade in the *Entolomataceae* using molecular DNA evidence (Baroni et al. 2011; Co-David et al. 2009). The morphological characters of this new species from North America clearly indicate it belongs in the genus *Entocybe.*

Materials & methods

Colors given are general color hues or matched to plates in Kornerup & Wanscher (1978). All microscopic structures were measured in mounts using 10% aqueous ammonia (household, non-sudsing, non-scented) or 3% KOH. Structures were also examined in mounts of Congo Red/ammonia, Melzer's Reagent and Cotton Blue/lactic acid to determine histochemical reactions of basidiospore and hyphal walls. For basidiospore measurements, the hilar appendix or apiculus was excluded. In those measurements Q refers to the length divided by the width of an individual spore. The notation n = 21 indicates that 21 individual spores were measured from a single sample. Means for length and width are given with their standard deviations, as is the mean of Q values, with the mean of the length divided by the width of all basidiospores measured being designated as Q^m.

Descriptive statistical analysis of basidiospores was determined using EXCEL 5.0. All light microscopic images were made with an Olympus BX 50 transmitted light microscope using DIC or bright field optics and captured with a Diagnostic Instruments, Inc. Insight Spot 3-shot color digital camera.

Scanning electron micrographs were captured on an ISI DS 130c scanning electron microscope run at 15 or 25 Kev. Tissue samples for SEM were prepared using a modified procedure originally described in Baroni (1981). Dried lamella fragments were soaked in 95% ETOH for 1 min, transferred to dH_2O for 1 min, then 3% KOH for 1 min, and finally washed three times in dH_2O for 1 min each. These rehydrated lamella fragments were then sandwiched between two filter papers in a modified Beem capsule and placed in 10% ETOH for a minimum of 2 minutes. The samples were dehydrated successively in 30, 50, 70, 80, 90, 95% ETOH solutions for 2 min each, and finally washed 2 more times in ice cold 100% ETOH before critical point drying in liquid CO_2 in a Tousimis Samdri PVT-3B critical point drier.

Results

The following new species is described from a single collection of multiple fruiting bodies found in the province of Québec, Canada. The large tricholomatoid stature and grayish yellow colors (Fig. 1) make the

Fig. 1. *Entocybe melleogrisea* (holotype). Basidiomata. Scale bar = 10 mm

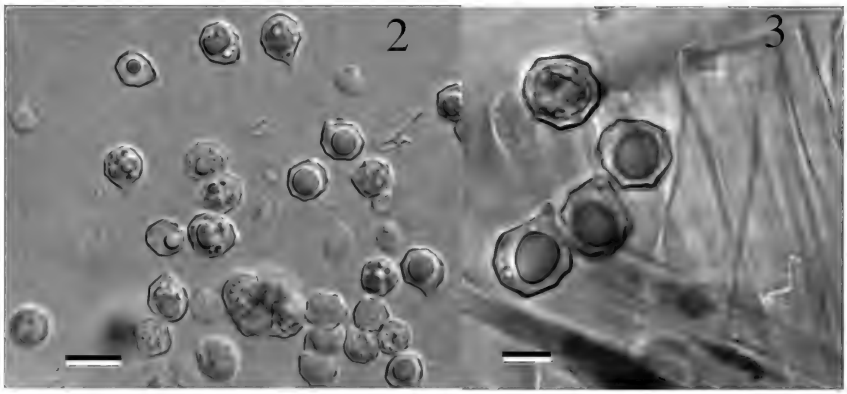
taxon distinctive in the field and unlike any other known species in North America. The subglobose or isodiametric basidiospores with obscure angles in profile and face views, the minute angles in polar view (Fig. 2), and the evenly cyanophilic walls of the basidiospores with the characteristic undulate-pustulate ornamentation (Fig. 3) coupled with obvious clamp connections in all tissues (Fig. 3) place this taxon in *Entocybe* (Baroni et al. 2011). The broken reticulum-like ornamentation (Fig. 4) as viewed under the scanning electron microscope is also a diagnostic feature for this group of temperate species of *Entolomataceae* (Baroni et al. 2011).

ITS (KC701386) and nLSU (KC701387; KC701388) sequences for our collection do not match any species in blast searches in GenBank. However the taxa that come closest in blast searches at 81–82% similarity are: *Entoloma nitidum* (AF335449.1 and AY228340.1), *Rhodocybe trachyospora* (JF899553.1), and *Entoloma turbidum* (FJ824815.1). These taxa are all considered members of *Entocybe* (Baroni et al. 2011), thus supporting our placement of this new species in that genus.

Entocybe melleogrisea T.J. Baroni & Y. Lamoureux, **sp. nov.** Figs 1–5

MycoBank MB 800629

Differing from other *Entocybe* species by the tricholomatoid stature, the grayish cream, opaque, non-hygrophanous, felted or fibrillose-squamulose pileus surface, the cream then pale brown stipe with a white, subbulbous, radicating base.



Figs. 2–3. *Entocybe melleogrisea* (holotype). **2.** Basidiospores. Scale bar = 10 µm. **3.** Basidiospores and clamp connection. Scale bar = 5 µm

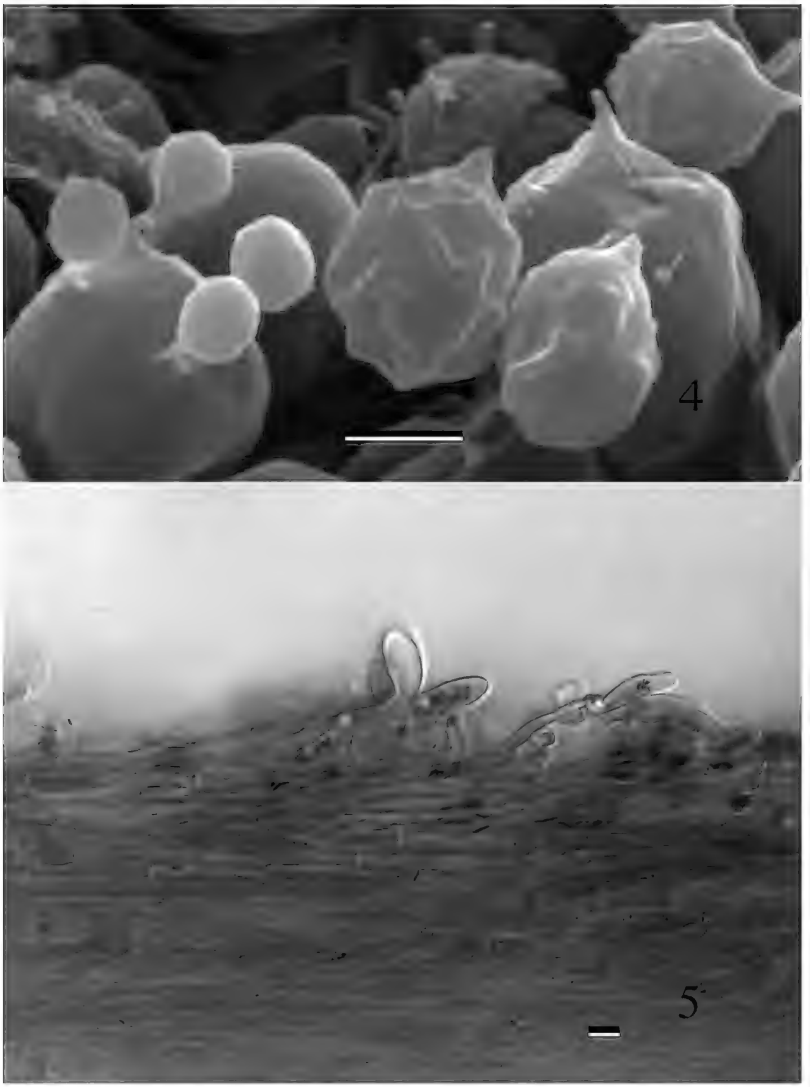
Type: Canada, Québec, City of Saint-Côme, scattered on soil and moss with dead leaves of *Betula papyrifera, Populus tremuloides,* and *Acer saccharum* or some fruiting directly from very rotten wood in a typical subboreal *Abies balsamea–Betula papyrifera* forest type of approximately 75 year old class, and also present in the area were *Picea* spp. and *Pinus strobus*, 18 August 2010, Yves Lamoureux 4150 (**Holotype**, CMMF; **isotype**, CORT). GenBank Accessions: KC701386 (ITS), KC701387 (nLSU, haplotype 1), KC701388 (nLSU haplotype 2).

Etymology: from the distinctive grayish cream pileus and stipe colors dominating or in later development as ground color.

Basidiomata tricholomatoid on soil or humus, subboreal forest.

Pileus 40–50 mm, grayish yellow, grayish cream, blond or grayish beige (4B-C3-6), or medium brown to dark blonde (5D4) over grayish cream ground color, sometimes paler toward margin or other areas (irregularly pigmented), convex to campanulate, surface not consistently smooth but irregular on some, becoming plane with no or a very small rounded umbo, surface finely felted, then finely fibrillose-squamulose toward margin, not viscid, not hygrophanous, but a few watery spots sometimes present, not striate, but margin clearly with fine raised lines on one basidioma (similar to species of *Leucopaxillus* Boursier and some of the brown *Tricholoma* (Fr.) Staude species); Context white, very thin (1 mm), fragile; Lamellae whitish then pinkish, adnexed then sinuate, close, moderately thick, very broad (up to 5 mm), edge even; Stipe 35–60 × 4–6(–8 at base) mm, cream then pale brown but white over base, striate and with appressed fibrils, equal but with base slightly bulbous and obliquely radicating, solid becoming stuffed, then hollow; Odor and Taste not distinctive or absent. Spore print dull pink (pale brownish pink).

Basidiospores (6.4–)7.2–8(–8.8) × (5.6–)6.4–7.2(–8) µm (x = 7.5 ± 0.54 × 6.9 ± 0.53 µm, Q = 1–1.23, Q^m = 1.09 ± 0.07, n = 21) isodiametric or



Figs. 4–5. *Entocybe melleogrisea* (holotype). **4.** SEM of basidiospores (note broken ridges and isolated pustules). Scale bar = 5 μm. **5.** Pileipellis with pilocystidia and intracellular pigments. Scale bar = 10 μm

subisodiametric, obscurely angular in profile view with 6–9 facets, isodiametric and obscurely angular in face view with 5–7 facets, minutely angled in polar view with 6–9 facets, undulate-pustulate under the light microscope in all views, walls evenly cyanophilic on smaller, younger spores and larger mature spores

with breached wall structure, otherwise wall of the larger spores not absorbing the dye, ornamentation composed of broken ridges and isolated pustules under an SEM; Basidia (2–)4-sterigmate, long clavate, hyaline, filled with refractive oil bodies, 39.7–44 × 10.5–12 µm; Hymenial cystidia absent; Lamella trama a hyaline layer of parallel, cylindrical or inflated hyphae, 3–13.8 µm in diam; Pileipellis (Fig. 5) a brown layer of ± repent, cylindrical hyphae producing scattered mounds or clusters of ± ascendant, entangled cylindrical, subclavate or clavate pilocystidia, 6–16 µm in diam, brown pigments intracellular and often coagulated into discrete masses of various sizes; Pileus context hyaline, composed of radially arranged cylindrical and inflated hyphae, 6–24 µm in diam; Stipitipellis similar to pileipellis, a compact brown layer of repent, cylindrical hyphae, 2–6 µm in diam, producing scattered mounds or clusters of entangled, ascendant cylindrical, subclavate or clavate end cells similar to the pileipellis, brown pigments intracellular, frequently coagulated; Clamp connections present in all tissues, large and obvious.

Discussion: The distinctive basidiospore morphology and presence of obvious large clamp connections on hyphae of the basidiomata place this taxon in *Entocybe*. From the senior author's experience, large obvious clamp connections are not typical in the *Entoloma* s.l. clade and thus seem to be another diagnostic feature that can be used to recognize *Entocybe* species. The brownish pink isodiametric basidiospores that are obscurely angled in profile view but possess 6–9 minute angular facets in polar view and pustule-like ornamentation are diagnostic for *Entocybe* (Baroni et al. 2011). Also for *Entocybe* species the ultrastructure of the basidiospore ornamentation is consistent and composed of a bumpy pustulate ornamentation partially interconnected with an incomplete network of ridges connecting at least some pustules. This incomplete reticulum is best seen under a scanning electron microscope (Fig. 4). Finally, as noted in the Results section, ITS sequences also appear to support the placement of *E. melleogrisea* with other like taxa in *Entocybe* and confirm nothing like it occurs in GenBank.

Molecular analyses (Baroni et al. 2011, Co-David et al. 2009) show that *Rhodocybe* species in the *Rhodocybe*/*Clitopilus* clade that also have clamp connections present on the hyphae, e.g., *Rhodocybe nitellina* and *R. melleopallens,* produce very differently shaped and ornamented basidiospores. The basidiospores are ellipsoid or amygdaliform and with scattered non-connected pustules or rounded bumps. *Entocybe* (Baroni et al. 2011) belongs in the *Entoloma* clade, not the *Rhodocybe*/*Clitopilus* clade of the *Entolomataceae,* and these morphological features emphasized for the genus are consistent.

Entocybe melleogrisea is somewhat similar to *E. turbida* (Fr.) T.J. Baroni et al. and *E. pseudoturbida* (Romagn.) T.J. Baroni et al. because of the tricholomatoid habit. Both *E. turbida* and *E. pseudoturbida* differ from *E. melleogrisea* in

having brown or grayish brown colors on the pileus and stipe, lacking yellow or cream hues, and the pilei for both are hygrophanous and thus changing color with loss of moisture. *Entocybe turbida* has a translucent-striate moist and mostly glabrous pileus surface except for the disc, which can be subtomentose, while *E. pseudoturbida* may be translucent-striate at the margin, but the pileus is subviscid and opaque when moist. Both species also produce farinaceous odors and taste (or a rancid taste in the case of *E. turbida;* Noordeloos 1992). A few collections of *E. turbida,* or something very similar to that taxon, have been found in southern Québec and in the southeastern US; however *E. pseudoturbida* has not yet been reported for North America.

Due to the felted opaque pileus surface and tricholomatoid habit, *E. melleogrisea* superficially reminds one of a member of the genus *Trichopilus* (Romagn.) P.D. Orton. However, for *Trichopilus* species the pileus surface is distinctly tomentose and not felted, the basidiospores are ornamented with interconnected ridges, not broken ridges, and the pileipellis is a trichodermium, not a cutis. Members of *Trichopilus* frequently have hymenial cystidia, and these are often lecythiform on the lamella edge, whereas none of the taxa placed in *Entocybe* have been found to produce hymenial cystidia.

Key to *Entocybe* species

Of the 12 *Entocybe* taxa documented from the northern hemisphere, most occur in North America (Baroni et al. 2011). We are aware of at least two other undescribed taxa in North America that appear to belong in this genus, but at this time we have inadequate materials to produce scientific descriptions.

1a. Basdiomata with blue or bluish fuscous or violaceous or vinaceous colors on the pileus, lamellae and/or stipe 2

1b. Basidiomata with brown, gray, yellowish brown or grayish yellow or grayish cream colors on the pileus and often also the stipe, although the stipe may be white, but constantly lacking any blue, violaceous or vinaceous hue..... 5

2a (1a). Pileus grayish blue, stipe blue or concolorous with pileus except for yellow hues in the base, lamellae white at first (Noordeloos 1992) ..... *E. nitida*

2b. Pileus grayish brown or dark purplish brown or reddish brown or fuscous purplish, lacking yellow hues in the base of the stipe, lamellae more highly colored 3

3a (2b). Pileus purplish brown to reddish brown at first, lamellae bluish gray at first, stipe dark blue to bluish gray, pileus flesh deep blue or bluish gray or purplish blue or violaceous gray..... *E. trachyospora* var. *purpureoviolacea*

3b. Pileus grayish brown, lamellae pallid or grayish brown not with blue colors, pileus flesh not purplish or violaceous but may be concolorous with pileus surface 4

4a (3b). Stipe surface, lamellae and pileus context with vinaceous hues ..... *E. trachyospora* var. *vinacea*

4b. Vinaceous colors lacking in these tissues, pileus dark grayish brown but hygrophanous and becoming paler, lamellae pallid or grayish brown at first, context buff ..... *E. trachyospora* var. *griseoviolacea*

5a (1b). Lignicolous. 6
5b. On humus, mosses, leaf litter, but not lignicolous 7

6a (5a). Pileus tan or yellowish brown, stipe yellow or pale orange, end cells in pileipellis variously shaped as pilocystidia, hyphae of lamella trama and pileus context dextrinoid . . . . . *E. speciosa*
6b. Pileus "off white to pale buff" becoming a uniform tan, stipe off white, lacking pilocystidia, hyphae of lamella trama and pileus context not dextrinoid (Largent 1989). . . . . *E. lignicola*

7a (5b). Stature tricholomatoid, medium to large with pileus diameters reaching 50–70 mm, lamellae adnexed or deeply emarginated to almost free or sinuate 8
7b. Stature collybioid, medium to small basidiomata, pileus diameters reaching 40 mm, lamellae attachment more variable, finely adnexed with decurrent tooth or emarginate or sinuate or adnate 10

8a (7a). Pileus grayish cream or grayish yellow or dark blond to medium brownish, but with grayish cream ground colors, dry, not hygrophanous, surface felted or minute fibrillose-squamulose, stipe concolor, odor and taste absent . . . . . *E. melleogrisea*
8b. Pileus gray-brown or sepia or reddish brown or dark umber brown, hygrophanous and becoming paler, stipe gray-brown or nearly concolorous with the pileus, odor farinaceous or absent, taste farinaceous or rancid or mild 9

9a (8b). Pileus moist, obscurely to distinctly translucent striate to mid pileus, grayish brown or sepia or reddish brown, pallescent after losing moisture, smooth, glabrous or subtomentose on disc when dry, stipe pale grayish brown and often with yellow hues over base especially when bruised, odor absent or somewhat farinaceous, taste mild or distinctly rancid (Noordeloos 1992). . . . . *E. turbida*
9b. Pileus subviscid when moist, not translucent striate or only at very margin, dark umber brown or sepia but slightly pallescent after losing moisture, radially fibrillose or subrugulose on drying, stipe gray or grayish brown, odor and taste strongly farinaceous (Noordeloos, 1992) . . . . . *E. pseudoturbida*

10a (7b). Stipe grayish over apex, yellowish downwards, distinctly tapered, ± rooting and often flexuous. . . . . *E. vinacea*
(if stipe entirely grayish *E. vinacea* var. *fumosipes*, if stipe violaceous then *E. vinacea* var. *violeipes* – Noordeloos 1992)
10b. Stipe off white, orange gray or brownish orange or hazel or vinaceous buff, enlarged at base or equal and tapering upward, not rooting, not flexuous 11

11a (10b). Stipe off white, orange gray or brownish orange with age and handling, lubricous, pileus grayish brown or dark yellowish brown or dark brown, lubricous, caulocystidia often sphaeropedunculate or pedicellate-capitate . . . . . *E. trachyospora var. trachyospora*
11b. Stipe hazel or vinaceous buff becoming buff, dry, pileus umber or brown vinaceous on the disc, pale umber or hazel or grayish sepia elsewhere, dry, caulocystidia mostly clavate or cylindrical . . . . . *E. priscua*

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A new species of *Miriquidica* from China

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Abstract — A new lichen species, *Miriquidica yunnanensis,* is described from China. Conspicuous features are its yellowish white to grayish white thallus, black-green epihymenium, colorless to light brown hypothecium, and a unique chemistry. A key to the seven similar *Miriquidica* species is also provided.

Key words — Yunnan, *Lecanorales,* saxicolous, taxonomy

Introduction

The generic concept of *Miriquidica* (*Lecanoraceae*) was first formulated by Hertel & Rambold (1987). It is characterized by lecideine apothecia, Lecanora-type asci, simple ascospores without a perispore, filiform conidia, and the occurrence of miriquidic acid in most species. This widely distributed genus presently includes 26 species worldwide and usually occurs on acidic rocks (Hertel & Rambold 1987; Rambold & Schwab 1990; Timdal 1993; Owe-Larsson & Rambold 2001; Hertel & Andreev 2003; Andreev 2004; Nash et al. 2004; Fryday 2008; Fryday & Coppins 2008; Coppins & Aptroot 2008; Lendemer & Knudsen 2008; Øvstedal et al. 2009). In China, only one species, *M. complanata,* has been reported (Wei 1991).

During our study of the lichen flora of Mt. Laojun in Yunnan province, China, a species of *Miriquidica* new to science was found. Here we present a brief diagnosis, an extended description, and a key to seven similar species in the genus.

Materials & methods

The specimen was collected from Yunnan, China, and is preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University (SDNU). The morphological and anatomical characters of the specimen were examined using a stereo microscope (Nikon SMZ 745T) and a polarizing microscope (Olympus CX41). Lichen substances in the specimen cited were identified using the standardized thin

layer chromatography techniques (TLC) with C system (Orange et al. 2010). The morphological and anatomical photos of the specie were taken under an Olympus SZX16 stereomicroscope and an Olympus BX61 compound microscope with DP72 respectively.

Taxonomic description

Miriquidica yunnanensis Lu L. Zhang & X. Zhao, **sp. nov.** Figs 1–2

MycoBank MB 802482

Differs from all other *Miriquidica* spp. by its yellowish white to grayish white thallus, blackish-green epihymenium, colorless to light brown hypothecium, small ascospores, and unique combination of chemical characters.

Type: China. Yunnan province, Mt. Laojun, alt. 4000 m, on rock, 7 Nov. 2009, H.Y. Wang 20121132 (**Holotype**, SDNU).

Etymology: The specific epithet "yunnanensis" refers to Yunnan province, where this species was found.

Thallus crustose, areolate, dispersed on a conspicuous black prothallus; areoles roundish, slightly convex to convex, small, 0.2–0.6(–0.8) mm; upper surface yellowish white to grayish white, due to pigmented hyphae

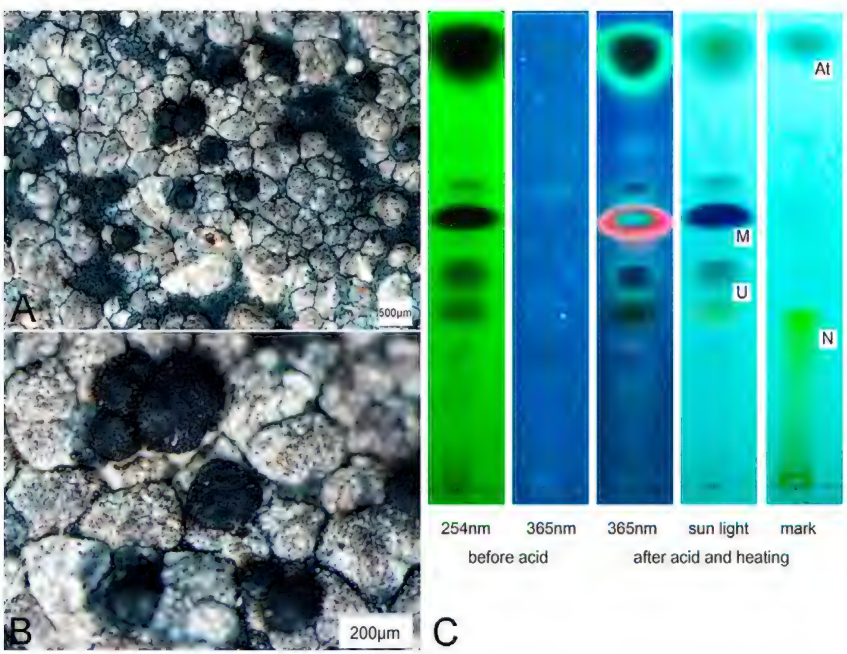

Figure 1. Miriquidica yunnanensis (Holotype: SDNU (Wang 20121132)). A, B. Thallus; C. TLC of chemical components (At = atranorin; M = miriquidic acid; N = norstictic acid; U = unknown substance).

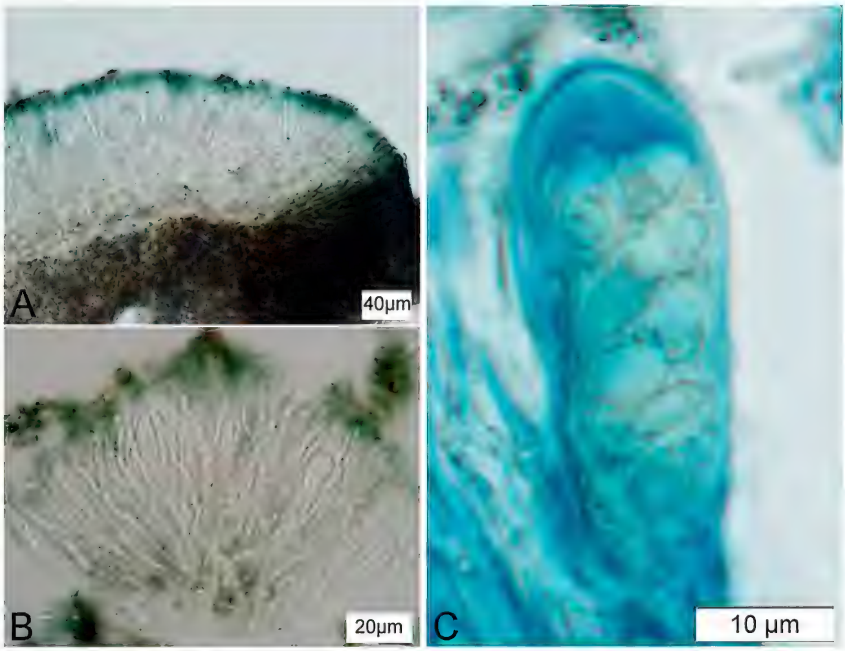

FIGURE 2. Miriquidica yunnanensis (Holotype: SDNU (Wang 20121132)). A. Section of apothecium; B. Paraphyses, after treatment with K; C. Ascus, after treatment with K/I.

of a lichenicolous fungus appearing dark gray, esorediate; MEDULLA I–, with some granules; PHOTOBIONT chlorococcoid; algal cells of 7–12.5(–15) μm in diam. APOTHECIA: sessile, 0.4–0.9 mm in diam.; DISC black, flat to convex, usually immarginate; EXCIPLE blackish green to black; EPIHYMENIUM blackish green; HYMENIUM hyaline, 60–70 μm; PARAPHYSES occasionally branched and anastomosing, 1.5–3 μm, apically 2–3.5 μm thick; HYPOTHECIUM hyaline to pale brown; ASCI of Lecanora-type s.l., 8-spored; ASCOSPORES hyaline, simple, ellipsoid to broadly ellipsoid, 8–11 × 5–6 μm. PYCNIDIA immersed, blackish; CONIDIA filiform, 10–15 μm long.

CHEMISTRY — Atranorin, miriquidic acid, norstictic acid, and unknown substances present.

DISTRIBUTION AND SUBSTRATE — Known only from Laojun Mountain, growing on siliceous rock at ca. 4000 m alt.

COMMENTS — Atranorin is not common in *Miriquidica;* only *M. atrofulva* (±) and *M. pycnocarpa* contain it in addition to *M. yunnanensis.* However, *M. atrofulva* is distinguished by a rusty colored thallus with dark blue-gray to blackish soralia and pale brown to olive-brown epihymenium and contains

stictic acid as a major compound; *M. pycnocarpa* differs in its I+ violet medulla, small apothecia (0.15–0.3 mm in diam.), and dark brown hypothecium and contains only atranorin.

Because *Miriquidica yunnanensis* is similar in morphology and anatomy to *M. disjecta, M. griseoatra, M. leucophaeoides, M. limitata,* and *M. paanaensis,* and *M. complanata* has been reported in China, we provide a key to these seven species.

Key to seven similar *Miriquidica* species

1a. Containing fumarprotocetraric acid..... *M. limitata*
1b. Lacking fumarprotocetraric acid 2
2a. Containing atranorin ..... *M. yunnanensis*
2b. Lacking atranorin 3
3a. Containing norstictic acid 4
3b. Lacking norstictic acid 5
4a. Thallus white, yellowish, or pale brown, glossy..... *M. leucophaeoides*
4b. Thallus pale gray to gray, tinged yellowish ..... *M. paanaensis*
5a. Thallus white to purely white..... *M. disjecta*
5b. Thallus gray, gray-brown or brownish yellow to dark bluish gray, matt 6
6a. Apothecia 0.2–0.7 mm diam., immersed..... *M. complanata*
6b. Apothecia 0.3–1.2 mm diam., sessile ..... *M. griseoatra*

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Lenzitopsis oxycedri (*Thelephoraceae, Basidiomycota*): newly recorded for the Balkan Peninsula

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Abstract — *Lenzitopsis oxycedri* is reported from the Balkan Peninsula for the first time, growing in a Mediterranean juniper forest in the Republic of Macedonia. This very rare species was collected on living branches of *Juniperus foetidissima* from Galichica National Park, in the southwestern part of the country.

Key words — wood-decay fungus, *Thelephorales*, stinking juniper

Introduction

Malençon & Bertault (1963) described *Lenzitopsis* as a monotypic genus in the *Thelephoraceae,* where it has an isolated position. *Lenzitopsis oxycedri* is characterized by perennial, resupinate to effuse reflexed basidiocarps, a monomitic hyphal system, and globose to subglobose spores that are finely warted and yellow to pale brown in KOH. Recently Zhou & Kõljalg (2013) described a second species in the genus, *Lenzitopsis daii* L.W. Zhou & Kõljalg, which differs by its annual basidiocarps, amyloid spores, and growth exclusively on *Juniperus chinensis* L.

The original description of *L. oxycedri* is based on a specimen growing on *Juniperus oxycedrus* L. in Morocco (Malençon & Bertault 1963). The first European records are from Guadalajara, Spain, where the species was found growing as a saprobe on *J. oxycedrus* and *J. thurifera* L. (Garcia-Manjon & Moreno 1981). Ryvarden (1991: 174) proposed *Lenzitella* and *L. malenconii* as replacement names for *Lenzitopsis* and *L. oxycedri*, which he mistakenly believed to be invalidly published; but because he included the valid Malençon & Bertault names in synonymy, both *Lenzitella* and *L. malenconii* are superfluous and

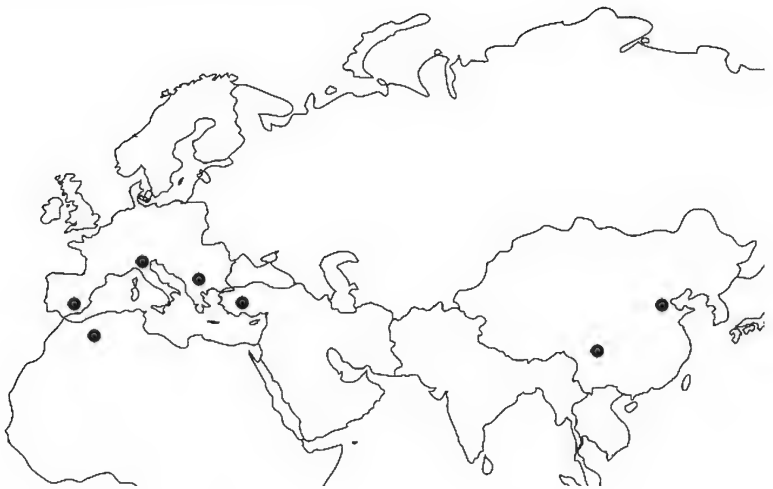


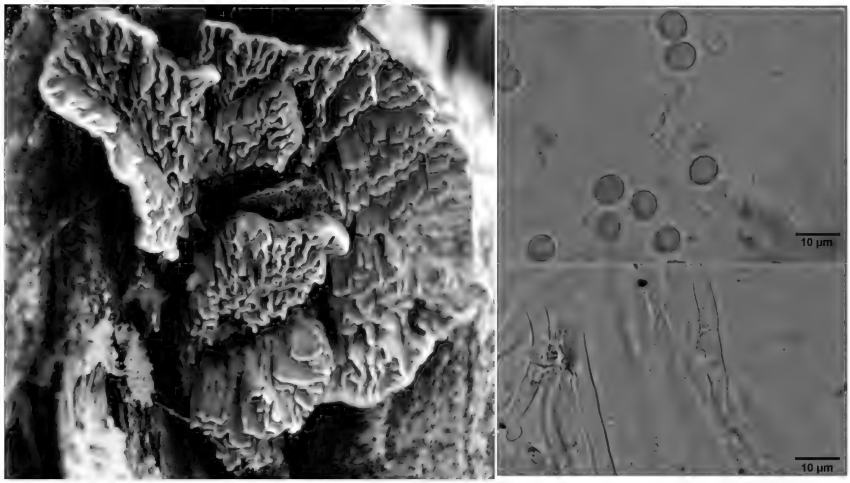
FIGURE 1. Geographical distribution of *Lenzitopsis oxycedri*.

illegitimate. Ryvarden's (1991) designation of a Spanish collection as "holotype" (to replace the original Moroccan material that is apparently lost) constitutes a neotypification of *L. oxycedri*. *Lenzitopsis oxycedri* has been reported from China (Dai et al. 2007), Italy (Bernicchia 2000), and Turkey, where it was collected twice in Thermessos National Park, Antalya, on branches of *Juniperus foetidissima* Willd. (Doğan et al. 2007). This paper extends the distribution to Macedonia, where *L. oxycedri* occurs on *J. foetidissima*.

Materials & methods

Collections were made during springtime (May) 2010 from *Juniperus–Quercus* forests in Galichica National Park, Republic of Macedonia. The forest primarily consists of *J. foetidissima* mixed with *Quercus* spp., but at certain spots *J. foetidissima* forms pure stands, sometimes intermixed with small stands of *J. excelsa* M. Bieb.

For identification we used the descriptions of Ryvarden & Gilbertson (1993: 376, as *Lenzitella malenconii*) and Bernicchia (2005). The nomenclature follows Index Fungorum (http://www.indexfungorum.org/names/Names.asp 2012) and Mycobank (http://www.mycobank.org/Biolomics.aspx?Table=Mycobank 2012). The samples were examined using classical methods. Sections were mounted in Melzer's reagent and in 5% aqueous solution of potassium hydroxide (KOH). Measurements and photographs were taken from sections mounted in KOH (5%) and examined at magnifications up to 1000× with an LW scientific microscope. Specimens are deposited in the Macedonian Collection of Fungi (MCF), within the Mycological Laboratory (Ss Cyril and Methodius University, Institute of Biology, Faculty of Natural Sciences and Mathematics).



Figures 2–4. *Lenzitopsis oxycedri* (MCF 10/11631). 2. Basidiocarp (left). 3. Spores (above right). 4. Generative hyphae with clamps (below right).

Taxonomy

Lenzitopsis oxycedri Malençon & Bertault, Bull. Soc. Mycol. Fr. 79: 82 (1963)

Figs 2–4

≡ *Lenzitella malenconii* Ryvarden, Syn. Fung. 5: 174 (1991), nom. illegit.

Type: Spain, Guadalajara, Tamajón, on living *Juniperus thurifera*, 26.4.1991, G. Moreno & L. Gonzaga (neotype, AH; isoneotype, O; Ryvarden 1991).

Basidiocarp perennial, effuse reflexed to pileate, projecting up to 2–3 cm wide, and 2–8 mm cm thick at base, corky to fragile when dry. Pileal surface dark brown, velutinate, azonate, later becoming glabrous and almost black. Hymenophore lenzitoid to lamellate, lamella 1–3 per mm, sinuous to raduloid or hydnoid with flat teeth, approximately 0.5 to 1 mm between the lamellae or spines, at first whitish, then ochraceous to pale brown. Context pinkish buff, corky, up to 2 mm thick, lamella concolorous with context, corky, up to 6 mm deep.

Hyphal system monomitic, generative hyphae with clamps, hyaline in trama and context, pale brown in tomentum, 3–5 µm wide, slightly thick-walled, sparingly branched, some irregular crystals present on hyphae. Cystidia absent.

Basidia with 4 sterigmata, at first clavate but elongating considerably to sinuous tube-like organs, 40–80 µm long, with a basal clamp. Basidiospores globose to subglobose, pale brownish, thick-walled, echinulate, often with small inner crystals of pigment, negative in Melzer's reagent, 5–6.9 × 4.9–5.9(–6.9) µm, L = 5.96 µm, W = 5.52 µm, Q = 1.08.

Specimens examined: **MACEDONIA, Galichica National Park:** Bitolski Pat, 1200 m a.s.l., *Juniperus foetidissima* forest with *Quercus* spp., at the base of branches of living *J. foetidissima*, 01.05.2010, Mitko Karadelev (MCF 10/11634); Zli Dol, 1400 m a.s.l., *J. foetidissima* forest with *Quercus* spp., at the base of branches of living *J. foetidissima*, 01.05.2010, Mitko Karadelev (MCF 10/11633); Velgoshti vill., 800 m a.s.l., pure *J. foetidissima* forest, at the base of branches of living *J. foetidissima*, 01.05.2010, Mitko Karadelev (MCF 10/11631).

Discussion

Macroscopically *Lenzitopsis oxycedri* resembles *Gloeophyllum sepiarium* (Wulfen) P. Karst. and *Daedaleopsis tricolor* (Bull.) Bondartsev & Singer in having a lamellate hymenophore. *Gloeophyllum sepiarium* most commonly grows on conifers and is yellowish to rusty brown in color, whereas *D. tricolor* develops on broadleaved trees, especially *Salix*. Both species have a dimitic hyphal structure and smooth basidiospores.

Since *L. oxycedri* is known from only a few localities in five Mediterranean countries and China (Figure 1), it is considered a very rare white rot saprobe or parasite that is apparently restricted to junipers. The Chinese specimens were collected from living junipers in Beijing and Sichuan Provinces (Dai et al. 2007), while elsewhere it occurs on *J. oxycedrus* in Morocco and Italy (Malençon & Bertault 1963, Bernicchia 2000, 2005), *J. oxycedrus* and *J. thurifera* in Spain (Garcia-Manjon & Moreno 1981), and *J. foetidissima* in Turkey (Doğan et al. 2007). Our specimens were also collected from *J. foetidissima* at three different sites within Galichica National Park in southwestern Macedonia. *Lenzitopsis oxycedri* may well prefer *J. foetidissima* in the eastern Mediterranean (Macedonia and Turkey), for it is found exclusively on *J. foetidissima* even in mixed juniper forests containing both *J. excelsa* and *J. foetidissima*.

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The authors gratefully thank Drs. H.H. Doğan (Selçuk University, Turkey), H. Kotiranta (Finnish Environment Institute, Helsinki), and Nomenclature Editor S.R. Pennycook for helpful presubmission reviews.

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Cortinarius of California: eight new species in subg. *Telamonia*

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Abstract — Described are eight new species of *Cortinarius* subg. *Telamonia* from California and the Pacific Northwest of North America. Four species fruit in the fall and the other four are vernal. *Cortinarius cisqhale* is a tanoak associate that fruits abundantly in the fall and winter. *Cortinarius athabascus* is an autumnal fruiting species and is broadly distributed throughout the region in conifer and mixed woods. *Cortinarius tuolumnensis*, *C. miwok*, *C. gualalaensis*, and *C. ohlone* belong to the /decipiens clade — the first two fruit vernally in boreal and montane habitat while the other two are autumnal at low elevations under coastal pine and live oak, respectively. *Cortinarius eldoradoensis* is a vernal fruiting species abundant in the higher elevations near melting snow. *Cortinarius truckeensis* is a rare, but locally abundant, species known only from its type location near Truckee, California.

Key Words — *Cortinariaceae*, fungal taxonomy, nrITS data

Introduction

Cortinarius subgenus *Telamonia* (Fr.) Trog is well represented in the state of California and the broader North American west. This geographical region features two distinct fruiting periods for *Cortinarius* — autumn, which is typical for the genus worldwide, followed by a rich vernal crop, predominantly in the boreal and montane areas during or shortly after snowmelt. The species composition is quite different between these fruiting periods with almost no overlap; only a few species are known to fruit in both seasons. This suggests that a long evolutionary path of adaptation to seasonal ecological factors has influenced the time of fruiting. While the vernal fruiting phenomenon for *Cortinarius* is not uncommon in Europe, it occurs far more frequently in the arid mountains of western North America.

Subgenus *Telamonia* is poorly studied in California, with only a handful of species reported or described. I am involved in a multi-year study of *Cortinarius* in California and the adjacent subcontinent, supported by extensive molecular

data analyses. Among the number of undescribed species that have emerged are eight common, well-understood taxa in subg. *Telamonia* described here.

Materials & methods

Methods for morphological studies and DNA extraction, PCR conditions and primers, PCR product clean-up, and sequencing follow Bojantchev & Davis (2011). Collections are stored in the author's private herbarium or at the University of California herbarium in Berkeley (UC) where noted. Extensive iconography of all newly described species is available on http://www.mushroomhobby.com. Tree genera are abbreviated with their initial letter, with the exception of *Pi.* = *Picea*; *P.* = *Pinus*; *Ps.* = *Pseudotsuga*.

Phylogenetic analysis—All *Cortinarius* nrDNA sequences from the public databases GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/) were downloaded and reviewed. A representative sample of 205 *Telamonia* sequences from the northern hemisphere (including 72 sequences from the author's collections) were selected for phylogenetic analysis based on whether they [1] provided good clade delineation throughout the entire subgenus or [2] represented taxa closest to the new species described here.

Sequence alignments were generated with MAFFT v6.821b (Katoh et al. 2002) with the G–INS–i global alignment iterative refinement strategy. Minimal gap opening and extension penalties were set for better resolution of the more variable sectors within the nrITS. The alignments were visually inspected and corrected where needed.

Phylogenetic reconstruction utilized Bayesian inference (BI) running on Mr.Bayes v.3.1.1 (Huelsenbeck & Ronquist 2003) with the General Time Reversible substitution model plus gamma distribution (GTR + Γ) as the best fit recommended by MrModeltest v.2.3 (Posada & Crandall 1998). The BI ran two independent analyses with four chains for 16,000,000 generations with sampling frequency for every 100th generation and a burnin value set at 400,000 (25%). The lowest average standard deviation of split frequencies achieved was 0.008821. A 50% majority rule consensus tree (Fig. 1) was generated with the posterior probability (PP) values showing above or below the branches.

Additional phylogenetic analysis of 38 sequences in the /decipiens clade (with *Cortinarius athabascus* as an outgroup), including all in GenBank that have ≥98% BLAST similarity index to *C. tuolumnensis*, *C. miwok*, *C. gualalaensis* and *C. ohlone*, was inferred using the Maximum Likelihood method based on the Tamura-Nei model as implemented by MEGA5 (Tamura et al. 2007). The resulting tree (Fig. 20) offers a much more detailed view of the /decipiens clade within Western North America, confirming the phylogenetic position of the newly described species as well as illuminating some commonly collected undescribed members of the clade.

Fig. 1. Phylogram generated by Bayesian inference of 205 *Cortinarius* subg. *Telamonia* nrITS sequences from the northern hemisphere. Two independent analyses with four chains for 16,000,000 generations were run with sampling frequency for every 100th generation and a burnin value set at 40,000 (25%). The posterior probabilities (PP) of the 50% majority rule consensus tree are showing above or below the branches. The branches of the new species described here are colored in red. The GenBank accession numbers are presented after the species names and the author's collection numbers are enclosed in parentheses. Holotype collections are marked by an asterisk. Misapplied species names on GenBank collections are enclosed in quotes.

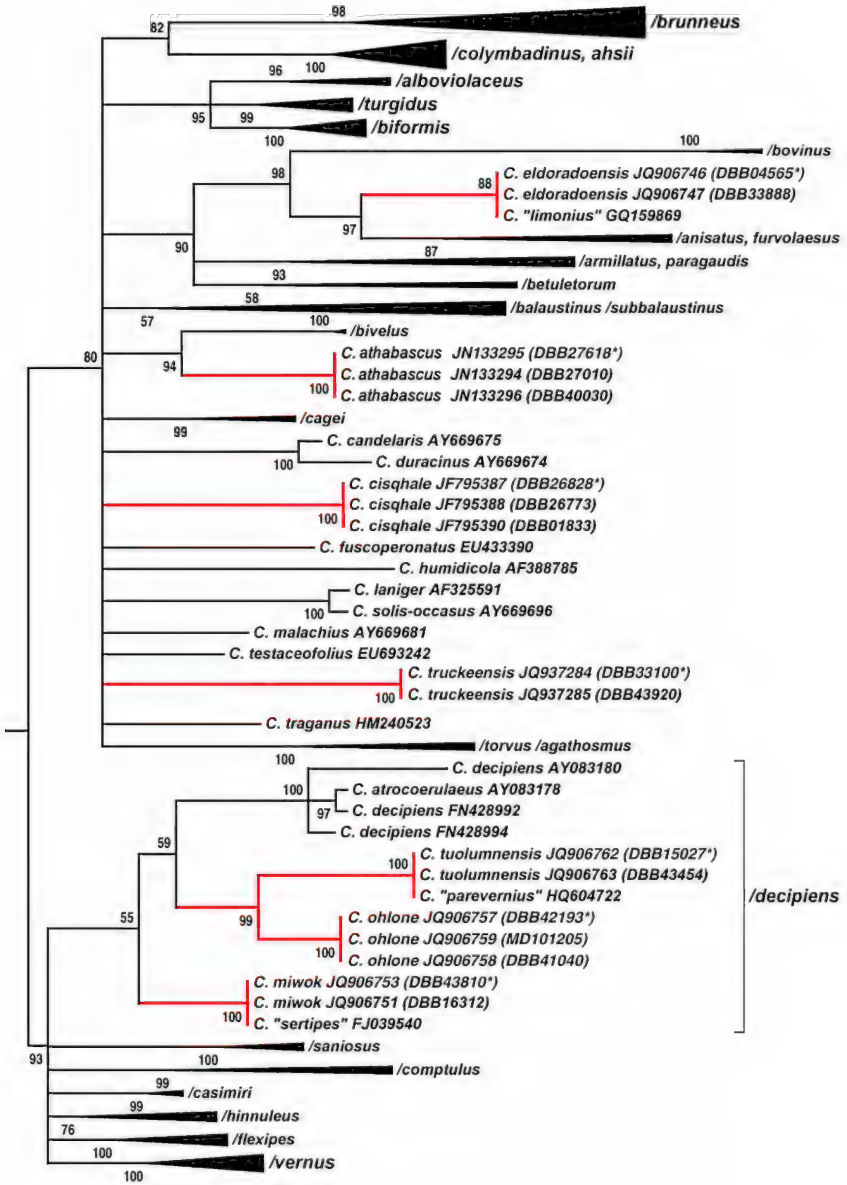
/brunneus
/colymbadinus, ahsii
/alboviolaceus
/turgidus
/biformis
/bovinus
C. eldoradoensis JQ906746 (DBB04565*)
C. eldoradoensis JQ906747 (DBB33888)
C. "limonius" GQ159869
/anisatus, furvolaesus
/armillatus, paragaudis
/betuletorum
/balaustinus /subbalaustinus
/bivelus
C. athabascus JN133295 (DBB27618*)
C. athabascus JN133294 (DBB27010)
C. athabascus JN133296 (DBB40030)
/cagei
C. candelaris AY669675
C. duracinus AY669674
C. cisqhale JF795387 (DBB26828*)
C. cisqhale JF795388 (DBB26773)
C. cisqhale JF795390 (DBB01833)
C. fuscoperonatus EU433390
C. humidicola AF388785
C. laniger AF325591
C. solis-occasus AY669696
C. malachius AY669681
C. testaceofolius EU693242
C. truckeensis JQ937284 (DBB33100*)
C. truckeensis JQ937285 (DBB43920)
C. traganus HM240523
/torvus /agathosmus
C. decipiens AY083180
C. atrocoerulaeus AY083178
C. decipiens FN428992
C. decipiens FN428994
C. tuolumnensis JQ906762 (DBB15027*)
C. tuolumnensis JQ906763 (DBB43454)
C. "parevernius" HQ604722
C. ohlone JQ906757 (DBB42193*)
C. ohlone JQ906759 (MD101205)
C. ohlone JQ906758 (DBB41040)
C. miwok JQ906753 (DBB43810*)
C. miwok JQ906751 (DBB16312)
C. "sertipes" FJ039540
/decipiens
/saniosus
/comptulus
/casimiri
/hinnuleus
/flexipes
/vernus

FIG. 2. *Cortinarius cisqhale* (UC 1860822, holotype).

Taxonomy

Cortinarius cisqhale Bojantchev, **sp. nov.** FIGS 2–7

MYCOBANK MB 800343

Differs from other *Telamonia* species by its distinct coloration, small spores, and association with Tanoak.

TYPE: USA. California: Sonoma County, Salt Point State Park, 0.5 mi. North of Hwy 1 (38°33′48″N 123°18′27″W), elev. 300 ft, under *Notholithocarpus densiflorus, P. muricata, Ps. menziesii,* 20 Nov 2009, Bojantchev DBB26828 (**Holotype** UC 1860822; Genbank nrITS JF795387, RPB2 JQ906739).

ETYMOLOGY: Cíšqhale (= beautiful tree) is the word for tanoak in the language of the Kashaya/Pomo Native American people.

PILEUS 40–120 mm diam., very variable in shape, hemispherical to convex when young, typically with a broad flattened umbo; margin involute then straight to flanging; colors diverse, mostly in the light to dark brown spectrum with grayish to olive tinges when wet, pale brownish when dry, often with bluish tinges outside of the disc, with the margins remaining whitish due to persistent velar remnants; surface smooth to shiny when wet, dull fibrillose when dry, hygrophanous in radial streaks, leaving irregular shapes, drying from the center out. LAMELLAE moderately crowded to subdistant, 5–18 mm broad, light or dark clay brown, occasionally lilac when young, turning rusty brown as the spores mature; edges even, pale; attachment sinuate; lamellulae abundant in series of 3–7. STIPE 50–120 mm long, 12–40 mm wide, cylindrical to subclavate, occasionally bulged at the base, frequently laterally subradicating,

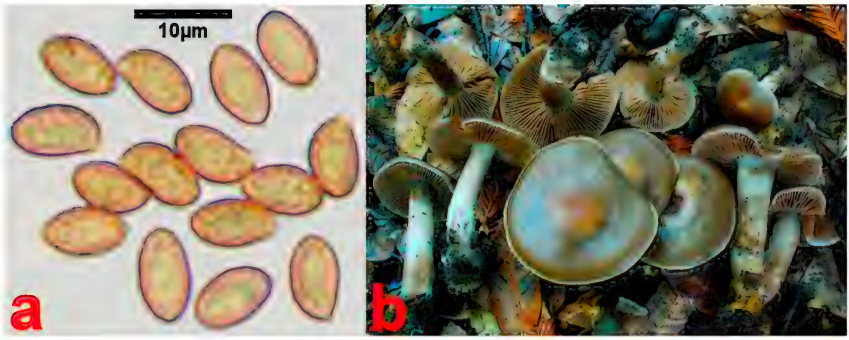

Fig. 3. *Cortinarius cisqhale* a) Basidiospores (UC 1860820, holotype) b) DBB27382

light to dark brown beneath the cottony white universal veil, with bluish tinges near the top on young basidiomata; mycelial strands occasionally present in mature basidiomata. Universal Veil gray white, covering the stipe over the entire length, often leaving an annular zone, easily bruised off. Cortina gray white. Context mottled, with grayish white zones mixed with brown in the lower stipe and with predominantly bluish tinges in the upper stipe; colors less contrasting at age, settling to grayish brown; bluish tinges stronger near the outer surface. Exsiccata dark gray to charcoal. Habit scattered to frequently caespitose of up to a dozen basidiomata. Odor earthy. Taste mild earthy. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (6.5–)7.0–8.0(–9.0) × (3.8–)4.0–4.6(–5.0) µm (mean 7.5 × 4.4 µm), Q = 1.59–1.86, Q_{av} = 1.73 (N = 198, 8 basidiomata, four collections), amygdaliform to narrowly ellipsoid, slightly ovate, moderately finely verrucose, non-dextrinoid. Basidia 28–36 × 5–7 µm, 4-spored, cylindro-clavate, clamped. Lamella edge sparsely fertile. Cystidia not observed. Lamellar trama composed of filamentous hyphae, 4–8 µm wide, with an olivaceous pigment. Pileipellis a cutis; upper layer composed of parallel hyphae, 4–12 µm wide, smooth, with olivaceous-brown pigment; lower layer hyphae 8–20 µm wide, thin walled. No distinct reaction with Melzer's reagent. Hypodermium not observed. Oleiferous hyphae common in all parts. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius cisqhale* appears to be associated solely with tanoak (*Notholithocarpus densiflorus*) and broadly distributed throughout its range. It has been collected along the California coast, in southern Oregon and the foothills of Sierra Nevada. At some locations in the fall it can be the most prevalent *Telamonia*. The only matching sequences in GenBank are from environmental studies on tanoak root tips (Bergemann & Garbelotto 2006).

Additional collections examined: **USA. California:** Mendocino County, Jackson State Demonstration Forest, off Little Lake Rd. (39°18′27″N 123°42′47″W), elev. 1800 ft, under *N. densiflorus, Ps. menziesii, Tsuga heterophylla, Sequoia sempervirens,* 14 Nov 2009, DBB26186; Feb 13, 2010, DBB27382, DBB27520; Jackson State Demonstration Forest, off Hwy 20. (39°21′29″N 123°38′11″W), elev. 1700 ft, under *N. densiflorus, Ps. menziesii, T. heterophylla, S. sempervirens,* Nov 24, 2007, DBB01833 (Genbank nrITS JF795390, RPB2 JQ906737); DBB01834; Nov 23, 2008, DBB09828, DBB10324; Sonoma County, Salt Point State Park, 0.5 mi. North of Hwy 1 (38°33′48″N 123°18′27″W), elev. 450 ft, under *N. densiflorus, P. muricata, Ps. menziesii,* 23 Nov 2007, DBB01378; 20 Nov 2009, DBB26773 (Genbank nrITS JF795388, RPB2 JQ906738), DBB26876, DBB27028; Yuba County, New Bullard's Bar Reservoir (39°25′23″N 121°05′27″W), elev. 2200 ft, under *N. densiflorus, P. ponderosa,* 24 Nov 2010, DBB39790 (Genbank nrITS JQ906745 RPB2 JQ906740); DBB40759; **Oregon:** Curry County, Samuel Boardman State Park, elev. 100 ft, under *N. densiflorus,* 11 Nov 2009, Bojantchev DBB27721.

Discussion — *Cortinarius cisqhale* is a very variable species with several distinct forms that could easily be interpreted as different species. Careful collecting and observation of basidiomata in all stages of development is required for the synthesis of a refined species concept. In some locales like the Sierra Nevada foothills and Santa Cruz this species tends to form clusters of long-stemmed fruitbodies in a narrowly caespitose habit (Fig. 6a). In wet conditions the coloration becomes distinctly olive-gray (Fig. 3b), while in dry conditions the collections look more like Fig. 4b. The material sometimes can be fairly dark (Fig. 1), while in age it fades to develop a strongly hygrophanous appearance (Fig. 7a,b). The exsiccata have a distinctly blackish-charcoal appearance, similar to *C. brunneus* (Pers.) Fr. and can easily be confused for a member of that clade. The distinctly mottled stem and persistent bluish-grey context coloration in the upper stem context are good taxonomic characters for identifying *C. cisqhale* in the field. A key factor is also the presence of tanoak.

While the nrITS gene region is the primary fungal barcode marker in the *Cortinarius* study used throughout this paper, the diversity of forms in *C. cisqhale* prompted using nrRPB2, an additional gene region that previous analysis of multiple collections (not shown) demonstrated a good species level resolution. The sequences of all examined *C. cisqhale* collections matched 100% in both gene regions.

Cortinarius cisqhale is quite isolated from any other sequences in GenBank, with the BLAST max identity index to the nearest neighbor 94% for nrITS and 92% for RPB2. The position of *C. cisqhale* within subg. *Telamonia* was not easily resolved as different methods and packages for phylogenetic reconstruction generated somewhat conflicting results (data not shown). The Bayesian analysis generated the most likely ancestral relationship scenario showing *C. cisqhale* as fairly isolated from most other known species. Future research will undoubtedly shed additional light on its ancestral hierarchy as more closely related species are found.

FIG. 4. *Cortinarius cisqhale* a) DBB39790 b) DBB27028

FIG. 5. *Cortinarius cisqhale* a)DBB09828 b) DBB10324

FIG. 6. *Cortinarius cisqhale* a) DBB40759, caespitose habit b) DBB27520

FIG. 7. *Cortinarius cisqhale* older, dry basidiomata a) DBB01833 b) DBB01834

FIG. 8. *Cortinarius athabascus* DBB27073.

Cortinarius athabascus Bojantchev, **sp. nov.** FIGS 8–11

MYCOBANK MB 800344

MISAPPLIED NAME – "*Cortinarius brunneus*" sensu Trudell & Ammirati (2009:151).

Differs from other *Telamonia* species by its distinct coloration, conifer association, autumnal fruiting, and western American distribution.

TYPE: USA. California: Mendocino County, Mendocino, off Crestwood Dr. (39°18′18″N 123°47′26″W), elev. 400 ft, under *P. muricata,* 21 Nov 2009, Bojantchev DBB27618 (**Holotype** UC 1860905; Genbank nrITS JN133295).

ETYMOLOGY: In honor of the Athabascan family of Native American tribes — this species can be found throughout its range along the Pacific coast of North America.

PILEUS 30–150 mm diam., campanulate to convex with 1–3 concentrically raised and depressed zones, typically with a broad flattened umbo; margin involute then straight, often flanging; dark brown with bluish-grey tinges when moist, drying to brown, or pale brown at age, often hygrophanous in concentric zones or radial streaks; veil remnants present at the extreme margin; surface smooth, innately fibrillose, dull when moist, often shiny when dry. LAMELLAE moderately crowded to subdistant, 6–22 mm broad, clay brown, turning rusty brown as the spores mature; edges even to slightly uneven; attachment sinuate; lamellulae abundant in series of 3–5. STIPE 50–180 mm long, 15–50 mm wide, cylindrical to slightly subclavate, often twisted, pale brown beneath the white veil remnants, bluish tinges at the extreme apex often preserved into maturity. UNIVERSAL VEIL white, abundant, covering the stipe over the entire length, often leaving a distinct annular zone, occasionally shedding white floccose scales on the pileus that disappear at maturity. CORTINA white. CONTEXT mottled with white to brown vertical streaks in stipe, with predominantly

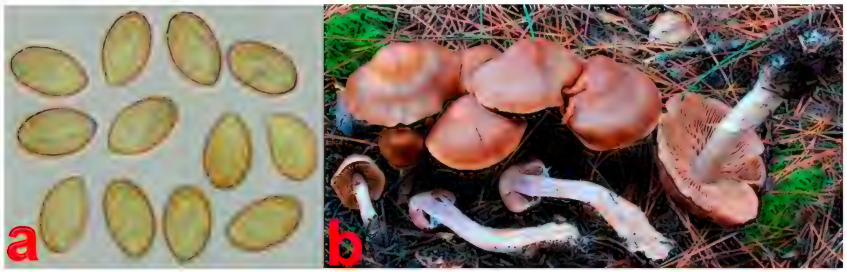

Fig. 9. *Cortinarius athabascus* (UC 1860905, holotype) a) Basidiospores b) Basidiomata

bluish tinges in the upper stipe and pronounced orange brown spots at the base. Exsiccata dark brown, the stipe brown. Habit mostly scattered, but frequently caespitose. Odor earthy. Taste mild earthy. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (8.0–)8.5–10.0(–10.5) × (5.0–)5.4–5.8(–6.3) µm (mean 9.2 × 5.6 µm), Q = 1.51–1.76, Q_{av} = 1.65 (N = 138, 5 basidiomata, four collections), amygdaliform to narrow ellipsoid, finely verrucose, non-dextrinoid. Basidia 27–35 × 5–8 µm, 4-spored, cylindro-clavate, clamped. Lamella edge infertile. Cystidia not observed. Lamellar trama composed of filamentous hyphae, 3–8 µm wide. Pileipellis a cutis, upper layer composed of thin parallel hyphae, 3–6 µm wide, brownish pigmented in KOH, walls smooth to very finely encrusted. Lower layer hyphae 5–18 µm wide, with thick hyaline walls. No distinct reaction to Melzer's reagent. Hypodermium not observed. Oleiferous hyphae sparse. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius athabascus* is widely distributed in northern coastal California and the Pacific Northwest in conifer and mixed woods. It has often been collected in the woods along the northern California coast, Oregon, and the Olympic Peninsula of Washington. There are several matching collections in GenBank, one of which (FJ717542) from Washington state is featured in Trudell & Ammirati (2007) as *C. brunneus*. This species has not been reported yet from the inner mountain ranges of western North America.

Additional collections examined: **USA. California**: Mendocino County, Mendocino, Jackson State Demonstration Forest, off Little Lake Rd. (39°18′27″N 123°42′47″W), elev. 100 ft, under *Ps. menziesii, N. densiflorus, T. heterophylla, S. sempervirens,* 21 Nov 2009, Bojantchev DBB27073; Gualala, off Old State Hwy (38°46′07″N 123°31′29″W), elev. 300 ft, under *Ps. menziesii, N. densiflorus, S. sempervirens,* 26 Nov 2010, Bojantchev DBB40030 (Genbank nrITS JN133296); Sonoma County, Salt Point State Park, 0.5 mi. North of Hwy 1 (38°33′48″N 123°18′27″W), elev. 300 ft, under *N. densiflorus, P. muricata, Ps. menziesii,* 23 Nov 2007, DBB01290 (Genbank nrITS JN133298); 20 Nov 2009, DBB26792 (Genbank nrITS JN133293), DBB27010 (Genbank nrITS JN133294); Humboldt County, Eureka (40°49′48″N 124°10′45″W),

Fig. 10. *Cortinarius athabascus* a) DBB40030 b) DBB40030 c) DBB01290

Fig. 11. *Cortinarius athabascus* a) DBB27010 b) DBB27010 b) DBB26792, a paler form

elev. 100 ft, under *Pi. sitchensis,* 20 Nov 2010, DBB39388; **Oregon**: Curry County, Samuel Boardman State Park, elev. 100 ft, under *Pi. sitchensis,* 11 Nov 2009, Bojantchev DBB37325; **Washington**: Clallam County, Olympic Peninsula, off Hot Spring Rd. (48°04′29″N 123°57′18″W), elev. 1000 ft, under *Pi. sitchensis, Pi. engelmannii, Ps. menziesii,* 21 Oct 2009, DBB24697;

Discussion — *Cortinarius athabascus* is one of the most common *Telamonia* species in the coastal woods of the Pacific rim of North America. It is also one of the larger *Telamonia* in the area, as it can achieve an impressive size. This species is quite variable in appearance with diverse hygrophanous patterns on the cap, while some forms remain quite pale even when moist. One of the key taxonomic differentials against other local *Telamonia* taxa is the long lasting and very distinct bluish coloration of the stem context in its upper part, even in mature basidiomata. The stepped/depressed multiple rings on the cap with smooth to innately fibrillose surface are good field characteristics too. Based on GenBank records this species is very commonly misidentified in the PNW as various European taxa. Careful analysis of the images presented in this study will help researchers to better comprehend the range of coloration and stature of this common western North American species.

Cortinarius eldoradoensis Bojantchev, **sp. nov.** Figs 12–15

MycoBank MB 800346

Differs from other *Telamonia* species by the totality of its coloration, montane vernal fruiting habit, and absence of fluorescent universal veil remnants.

Fig. 12. *Cortinarius eldoradoensis* DBB15611. Note the distinct cavities in the stipe.

Type: USA. California: El Dorado County, El Dorado National Forest, 0.1 mi. east of Wrights Lake Road (38°48′20″N 120°14′18″W), elev. 6100 ft, under *P. ponderosa, Abies concolor, A. magnifica, Ps. menziesii,* 26 May 2007, Bojantchev DBB04565 (**Holotype** UC 1861350; Genbank nrITS JQ906746).

Etymology: after the Eldorado National Forest in the Central Sierra Nevada.

Pileus 50–120 mm diam., convex to plano-convex without umbo; margin involute, frequently flanging at age; brown with bluish grey tints towards the disk; the extreme margin frequently whitish due to velar remnants; surface smooth to glossy, innately fibrillose, inconspicuously hygrophanous with occasional radial streaks. Lamellae moderately crowded, 7–22 mm broad, latte brown at first, turning rusty brown as the spores mature; edges even, attachment sinuate; lamellulae abundant. Stipe 40–80 mm long, 15–30 mm wide, cylindrical to subclavate, white to beige, sometimes shiny. Universal Veil white, partially covering the lower stipe, often leaving an evanescent annular zone. Cortina white. Context white to beige, developing cavities along the length of the stipe. Exsiccata dark brown. Habit scattered to sub-caespitose. Odor earthy. Taste mild earthy. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (8.0–)8.5–10.5(–11.3) × (4.8–)5.0–6.0(–6.5) µm (mean 9.5 × 5.5 µm), Q = 1.34–2.08, Q_{av} = 1.73 (N = 203, 7 basidiomata, five collections), variable in shape, mainly amygdaliform, but varying from ovoid to cylindro-amygdaliform to oblong-ellipsoid, moderately verrucose, non-dextrinoid.

Fig. 13. *Cortinarius eldoradoensis* a) DBB15638 b) UC 1861350, holotype

Fig. 14. *Cortinarius eldoradoensis* a) DBB33888 b) DBB15626

Basidia 26–36 × 6–9 µm, 4-spored, cylindro-clavate, clamped. Lamella edge sparsely fertile. Cystidia not observed. Lamellar trama regular, composed of more or less parallel, cylindrical hyphae, 2–8 µm wide. Pileipellis a cutis, upper layer composed of thin parallel hyphae, 3–5µm wide with pale olivaceous pigment in KOH, walls smooth, not encrusted. Lower layer hyphae 6–22 µm wide, with thin walls. No distinct reaction with Melzer's reagent. Hypodermium

Fig. 15. *Cortinarius eldoradoensis*
a) Oleiferous hypha in the pileus trama (UC 1861350, holotype)
b) Basidiospores (UC 1861350, holotype)

not developed. Oleiferous hyphae abundant in all parts, with thick refractive walls and olivaceous pigment. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius eldoradoensis* fruits abundantly in late spring and early summer in the mountains of California during, or soon after snow melt. Apparently it is broadly distributed in the Pacific Northwest, yet probably uncommon, as there is only one matching sequence in GenBank (GQ159869) from Mt. Washington, Vancouver Island, British Columbia (also vernally fruiting). In California this species is very common in both the foothills of the Sierra Nevada and the higher elevations of the Sierra–Cascade range where it can frequently be observed right along the banks of melting snow. The typical surrounding trees are conifers like ponderosa pine (*P. ponderosa*), white fir (*A. concolor),* red fir (*A. magnifica*) and Douglas-fir (*Ps. menziesii*).

Additional collections examined: **USA. California**: El Dorado County, El Dorado National Forest, 0.1 mi. East of Wrights Lake Road (38°48′20″N 120°14′18″W), elev. 6100 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 22 May 2009, DBB15611 (Genbank nrITS JQ906747); DBB15626; DBB15638; Tuolumne County, Yosemite National Park, off Hodgdon Meadow Access Rd (39°18′25″N 123°42′53″W), elev. 5100 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 25 Jun 2010, DBB33888 (Genbank nrITS JQ906748); DBB34062 (Genbank nrITS JQ906749); Sierra County, Chapman Creek Campground (39°37′54″N 120°32′37″W), elev. 5900 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 14 May 2010, DBB43454 (Genbank nrITS JQ906750);

Discussion — *Cortinarius eldoradoensis* is a typical member of the rich vernal snowbank *Telamonia* biota of the Sierra–Cascade range. Most of these species share similar brownish coloration and overall stature, which makes them difficult to separate in the field. The problem is exacerbated by the fact that these species fruit in moist spots near melting snow where the soil is very soft and obtaining clean material is difficult. Several years of field observation of multiple collections were needed in order to understand this species better. There is a cluster of several very similar, undescribed, vernal species, which can be separated based on their positive UV fluorescence. The less similar vernal species in the /decipiens clade, like *C. miwok* and *C. tuolumnensis,* develop purplish coloration in the stipe context. In addition, most *C. eldoradoensis* basidiomata develop distinct cavities in the stipe, which can be diagnostic for this species.

Cortinarius truckeensis Bojantchev, **sp. nov.** Figs 16–19

MycoBank MB 800349

Differs from other *Telamonia* species by the distinct white coloration, montane vernal fruiting habit, and large spores.

Type: USA. California: Nevada County, North of Truckee, off Hwy. 89 (39°24′05″N 120°11′19″W), elev. 6200 ft, under *P. jeffreyi,* 12 Jun 2010, DBB33100 (**Holotype** UC 1861353; Genbank nrITS JQ937284).

FIG. 16. *Cortinarius truckeensis* (UC 1861353, holotype).

ETYMOLOGY: after the town of Truckee, California in the vicinity of which this species is abundant.

PILEUS 40–100 mm diam., convex to plano-convex to depressed; margin involute into maturity, often wavy; white to light gray-white, remaining so into maturity; surface fibrillose, smooth to shiny, inconspicuously hygrophanous. LAMELLAE moderately crowded to subdistant, 8–26 mm broad, tan to latte brown, without bluish tinges, turning rusty brown as the spores mature; edges even, paler than the sides; attachment sinuate; lamellulae abundant in series of 3–7. STIPE 30–90 mm long, 20–44 mm wide, cylindrical, white, occasionally with a silky shiny surface, solid well into maturity. UNIVERSAL VEIL white, occasionally leaving an evanescent annular zone at the base of the stipe. CORTINA white. CONTEXT white to pale beige, sometimes mottled with tan to brown longitudinal streaks. EXSICCATA brownish with silvery sheen, particularly on the stipes. HABIT subcaespitose to scattered. ODOR earthy. TASTE mild earthy. UV LIGHT no fluorescence detected. MACROCHEMICAL REACTIONS 5% KOH negative. SPORE DEPOSIT rusty brown.

BASIDIOSPORES (8.5–)9.0–11.2(–12.0) × (4.8–)5.0–5.5(–6.0) µm (mean 10.1 × 5.3 µm), Q = 1.70–2.13, Q_{av} = 1.91 (N = 146, 4 basidiomata, four collections), inequilaterally amygdaliform to broadly fusiform, some strangulated near the apex, very finely verrucose, non-dextrinoid. BASIDIA 32–44 × 7–10 µm, 4-spored (some 2-spored), cylindro-clavate, clamped. LAMELLA EDGE sterile. CYSTIDIA not observed. LAMELLAR TRAMA regular, composed of more or less parallel, cylindrical hyphae, 4–10 µm wide, with brown intracellular pigment when viewed in KOH. PILEIPELLIS a cutis, upper layer composed of thin parallel hyphae, 3–8 µm wide with brown pigment in KOH, walls smooth. Lower layer hyphae 9–25 µm wide, with thin walls. No distinct reaction with Melzer's reagent. HYPODERMIUM well developed. OLEIFEROUS HYPHAE infrequent.

Fig. 17. *Cortinarius truckeensis*
a) Basidiospores (UC 1861353, holotype) b) DBB16054, young basidiomata.

Clamp connections common on all septa.

Habitat and distribution — *Cortinarius truckeensis* is known only from its type location where it has fruited abundantly for several consecutive years. The habitat is composed almost solely of Jeffrey's Pine (*P. jeffreyi*). Despite the extensive field research and many organized forays in the Sierra Nevada, this species has never been collected outside of the type location. Typically, *C. truckeensis* fruits under a very thick conifer duff and rarely breaks above the surface of the forest floor. Most commonly the location of fruiting is marked by a hump that may hide a dozen or more clustered basidiomata.

Fig. 18. *Cortinarius truckeensis* a) DBB33200 b) DBB43920

Fig. 19. *Cortinarius truckeensis* mature basidiomata DBB43920 a) DBB43920 b) DBB33200

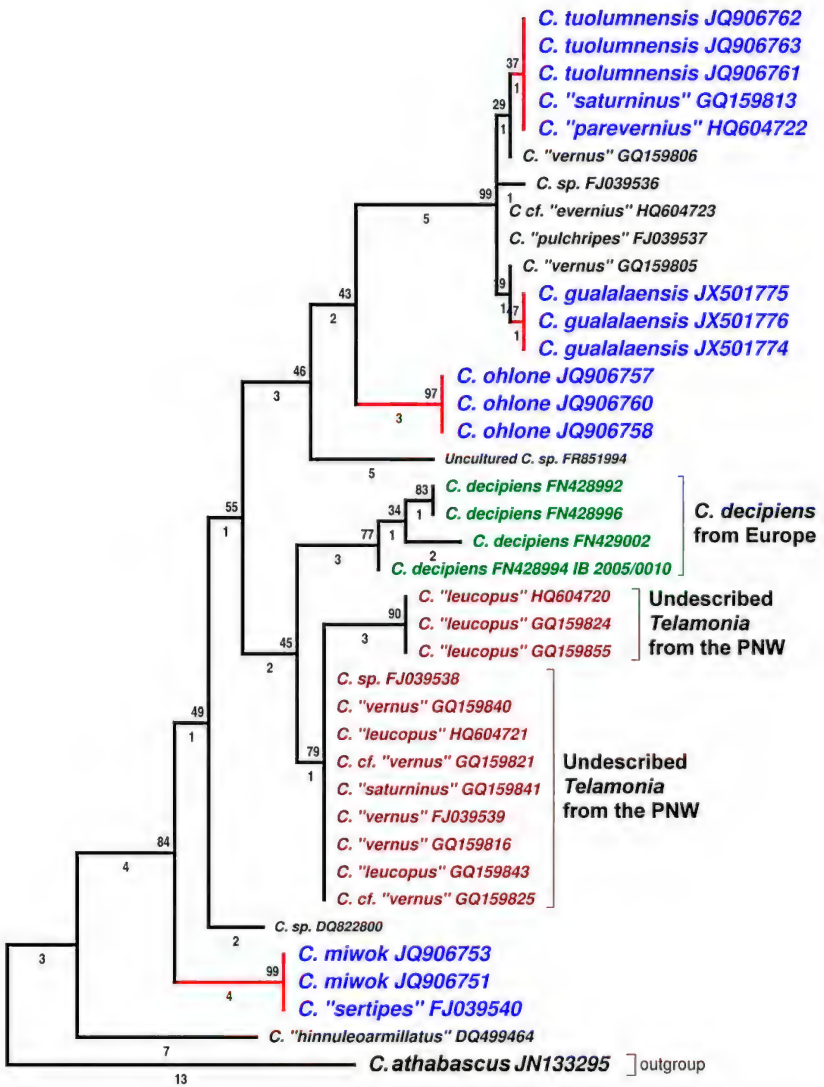

FIG. 20. Phylogenetic tree inferred by Maximum Likelihood analysis of 38 nrITS Cortinarius sequences in the /decipiens clade (with *C. athabascus* as an outgroup). The tree shows all GenBank sequences that are within 2% BLAST similarity index to *C. tuolumnensis*, *C. miwok*, *C. gualalaensis* and *C. ohlone*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches and the branch length values are shown below the branches. GenBank accession numbers are shown after the binomial. Misapplied species names on GenBank collections are enclosed in quotes.

Additional collections examined: **USA. California**: Nevada County, North of Truckee, off Hwy. 89 (39°24′05″N 120°11′19″W), elev. 6200 ft, under *P. jeffreyi*, 15 Jun 2006, DBB00054; 18 Jun 2009, DBB16054; 12 Jun 2010, DBB33200; 9 Jun 2011, DBB43920 (Genbank nrITS JQ937285);

Discussion — *Cortinarius truckeensis* is one of the most distinctive species in subg. *Telamonia*, and is one of the easiest to identify in the field as the combination of predominantly white coloration, habitat, and season of fruiting sets it apart. One of the unusual features of this species is the spore shape (Fig. 17a) and size that resemble those of the phylogenetically distant species in subg. *Phlegmacium* sect. *Variecolores* Brandrud & Melot.

This species seems somewhat isolated from any of the *Cortinarius* collections represented by sequence data in GenBank and even more so from those in the European public database UNITE. As with *C. cisqhale*, establishing the phylogenetic position of *C. truckeensis* presented difficulties as various packages and methods produced contradictory results. Neighbor-joining and maximum parsimony analyses suggested affinity towards either the /alboviolaceus or the /evernius clades. The Bayesian analysis suggests that this species is somewhat isolated on its own evolutionary path without known close relatives.

Species in the /decipiens clade

Cortinarius tuolumnensis Bojantchev, **sp. nov.** Figs 21–24

MycoBank MB 800345

Differs from other *Telamonia* species by the distinct context coloration, montane vernal fruiting habit, and spore shape.

Type: USA. California: Tuolumne County, Yosemite National Park, off Hodgdon Meadow Access Rd. (37°48′28″N 119°51′19″W), elev. 4600 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii*, 14 May 2009, DBB15027 (**Holotype** UC 1861347; Genbank nrITS JQ906762).

Etymology: after the Tuolumne river and county in the Central Sierra Nevada.

Pileus 20–90 mm diam., hemispherical to convex when young, plano-convex at age, often with a broad rounded umbo; margin involute then straight; blackish-brown when young and/or moist, lighter reddish-brown to chestnut-brown to cinnamon-brown at maturity or when dry; the disk and umbo remain darker, fading at age; bruising purplish-black, hygrophanous in radial streaks. Lamellae moderately crowded to subdistant, 5–12 mm broad, latte brown at first, turning rusty brown as the spores mature; edges slightly uneven, conspicuously lighter in color; attachment sinuate; lamellulae abundant in series of 3–5. Stipe 40–130 mm long, 6–15 mm wide, cylindrical, straight or curved due to caespitose habit, light brown beneath the universal veil, sometimes with grayish-blue tinges near the top; with longitudinal cavity. Universal Veil white, covering the stipe over the entire length, often leaving an annular zone,

Fig. 21. *Cortinarius tuolumnensis* (UC 1861347, holotype).

fibrous at first, sometimes glossy at age. Cortina white. Context beige with conspicuous purple-brown bruising in young material, often completely absent with age. Exsiccata dark brown, stipes brown. Habit often caespitose of up to a dozen basidiomata, but frequently scattered. Odor distinctly spicy with a hint of fruit (especially in young basidiomata). Taste mild. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (8.5–)9.5–11.2(–12.5) × (5.0–)5.7–6.5(–7.0) µm (mean 10.2 × 6.1 µm), Q = 1.52–1.88, Q_{av} = 1.67 (N = 153, 6 basidiomata, five collections), amygdaliform to narrow ellipsoid, slightly ovate, strongly verrucose, more so towards the apex, non-dextrinoid. Basidia 34–40 × 8–10 µm, 4-spored (frequently 2-spored), cylindro-clavate, clamped. Lamella edge sparsely

Fig. 22. *Cortinarius tuolumnensis* a) Basidiospores (UC 1861347, holotype) b) DBB00114

Fig. 23. *Cortinarius tuolumnensis* a) DBB09856 b) DBB43454 c) DBB00112

Fig. 24. *Cortinarius tuolumnensis* DBB00112
a) context discoloration in young basidiomata b) cortina c) clustered/caespitose habit

fertile. Cystidia not observed. Lamellar trama regular, composed of more or less parallel, cylindrical hyphae, 2–10 µm wide. Pileipellis a cutis, upper layer composed of thin parallel hyphae, 2–5 µm wide with red-brown pigment in KOH, walls smooth to finely zebra-stripe encrusted. Lower layer hyphae 6–22 µm wide, with thin walls. No distinct reaction with Melzer's reagent. Hypodermium well developed. Oleiferous hyphae none observed. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius tuolumnensis* fruits abundantly in late spring and early summer in the mountains of California and in the broader Pacific Northwest. There are two matching vernally fruiting collections represented in GenBank (HQ604722, HQ604723) from Victoria, British Columbia. It appears that the preferred habitat of this species in California is the wet montane meadows (soon after snow melt), surrounded by conifers, mostly pines like Ponderosa pine (*P. ponderosa*).

Additional collections examined: **USA. California**: El Dorado County, El Dorado National Forest, 0.1 mi. SE of Icehouse Road (38°51′24″N 120°22′32″W), elev. 5100 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 14 May 2011, DBB43454 (Genbank nrITS JQ906763); Tuolumne County, Stanislaus National Forest, off Evergreen Rd. (37°49′55″N 119°51′04″W), elev. 4500 ft, under *P. ponderosa, A. concolor, Ps. menziesii,* 13 May 2007, DBB00112 (Genbank nrITS JQ906761) and DBB00114; 22 May 2008, DBB09856.

Discussion — When first observed in the field, *C. tuolumnensis* was easily placed in the /decipiens clade (Fig. 20) due to the purplish discoloration on

the stipe context and distinct spicy odor. That position was confirmed later by the nrITS generated phylogeny. *Cortinarius tuolumnensis* stands out from other clade representatives (such as *C. miwok*) by its caespitose habit and typically longer stems.

FIG. 25. *Cortinarius gualalaensis* (UC 1998883, holotype).

Cortinarius gualalaensis Bojantchev, **sp. nov.** FIGS 25–27

MYCOBANK MB 801217

Differs from *C. tuolumnensis* by the autumnal fruiting habit and slightly smaller spores.

TYPE: USA. California: Sonoma County, Jenner, Salt Point Lodge, off Hwy 1. (38°33′24″N 123°18′10″W), elev. 120 ft, under *P. muricata,* 21 November 2010, DBB39900 (**Holotype** UC 1998883; Genbank nrITS JX501775).

ETYMOLOGY: after the coastal city of Gualala in northern California — the name means "where the river meets the sea" in the language of the Pomo tribe.

PILEUS 20–70 mm diam., hemispherical to convex when young, plano-convex at age, often with a broad rounded umbo; margin straight then flanging; dark-brown when young and/or moist, lighter reddish-brown to chestnut-brown to dull cinnamon-brown at maturity or when dry; the disk and umbo remain darker, fading at age; bruising purplish-black, strongly hygrophanous with fading radial streaks. LAMELLAE moderately crowded to subdistant, 4–15 mm broad, latte brown at first, turning rusty brown as the spores mature; edges slightly uneven, conspicuously lighter in color; attachment sinuate; lamellulae abundant in series of 3–5. STIPE 30–120 mm long, 5–14 mm wide, cylindrical, straight or curved due to caespitose habit, light brown beneath the universal veil remnants, sometimes with grayish-blue tinges near the top; with longitudinal cavity. UNIVERSAL VEIL white, covering the entire stipe, but more prominent in the lower portion, often leaving a faint annular zone, sometimes glossy in age.

Cortina white. Context beige with conspicuous purple-brown bruising in young material, often completely absent at age. Exsiccata dark brown, stipes lighter in color. Habit mostly caespitose of up to a dozen basidiomata, but frequently scattered. Odor somewhat spicy (especially in young basidiomata). Taste mild. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (8.5–)9.0–11.0(–11.5) × (5.8–)6.0–6.8(–7.3) µm (mean 9.9 × 6.5 µm), Q = 1.42–1.75, Q_{av} = 1.54 (N = 143, 5 basidiomata, three collections), amygdaliform to ellipsoid, distinctly verrucose, more so towards the apex, non-dextrinoid. Basidia 32–42 × 7–11 µm, 4-spored (frequently 2-spored), cylindro-clavate, clamped. Lamella edge sparsely fertile. Cystidia not observed. Lamellar trama regular, composed of more or less parallel, cylindrical hyphae, 3–12 µm wide. Pileipellis a cutis, upper layer composed of thin parallel hyphae, 2–6 µm wide with red-brown pigment in KOH, walls smooth to finely zebra-stripe encrusted. Lower layer hyphae 5–25 µm wide, with thin walls. No distinct reaction with Melzer's reagent. Hypodermium well developed. Oleiferous hyphae none observed. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius gualalaensis* fruits in the fall and is known only from the coastal areas of California and Oregon. In California, *C. gualalaensis* was collected in dense stands of Bishop pine (*P. muricata*) while in the coastal sand dunes of Oregon this species fruited under Shore pine (*P. contorta* var. *contorta*).

Fig. 26. *Cortinarius gualalaensis* (UC 1998883, holotype) a) Basidiospores b) Basidiomata

Fig. 27. *Cortinarius gualalaensis* a) DBB43121 b) DBB43121 c) DBB23436

Additional collections examined: **USA. California**: Sonoma County, Salt Point State Park, 0.5 mi. North of Hwy 1 (38°33′48″N 123°18′27″W), elev. 300 ft, under *P. muricata,* 27 Nov 2011, DBB43121 (Genbank nrITS JX501776); **Oregon**: Lane County, Jessie M. Honeyman Memorial State Park (43°55′48″N 124°06′26″W), elev. 80 ft, under *P. contorta* var. *contorta,* 9 Nov 2009, DBB23436 (Genbank nrITS JX501774);

Discussion — *Cortinarius gualalaensis* is very closely related to *C. tuolumnensis* and appears very similar in stature, colors, and the clustered habit. The major differentiating character is the time of fruiting — *C. tuolumnensis* fruits in the spring and *C. gualalaensis* in the autumn. Microscopically, *C. gualalaensis* spores are a bit shorter and wider, with a smaller Q_{av}.

Fig. 28. *Cortinarius ohlone* DBB41040.

Cortinarius ohlone Bojantchev, **sp. nov.** Figs 28–31

MycoBank MB 800348

Differs from other *Telamonia* by the unique coloration and association to oaks in California and adjacent regions.

Type: USA. California: Contra Costa County, Kennedy Grove (37°56′47″N 122°15′58″W), elev. 100 ft, under *Quercus agrifolia,* 23 Jan 2011, DBB42193 (**Holotype** UC 1861349; Genbank nrITS JQ906757).

Etymology: In honor of the Ohlone Native American people — this species can be found in the oak woods of northern California that they inhabited.

Pileus 30–80 mm diam., convex to plano-convex, rarely with a broad rounded umbo; margin more or less involute into maturity; dark brown to grey-brown. When young with dark bluish-gray cast, turning cinnamon brown at

Fig. 29. *Cortinarius ohlone*
a) Basidiospores (UC 1861349, holotype)
b) DBB05460, young basidiomata with dark bluish-gray cast on the pileus

maturity; surface fibrillose, smooth to shiny, inconspicuously hygrophanous with radial streaks. Lamellae moderately crowded to subdistant, 5–18 mm broad, dark clay brown, without bluish tinges, turning rusty brown as the spores mature; edges slightly uneven, much paler than the sides; attachment sinuate; lamellulae abundant in series of 3–7. Stipe 30–120 mm long, 12–24 mm wide, cylindrical, white to pale beige. Universal Veil white, partially covering the lower stipe, leaving an evanescent annular zone. Cortina white. Context beige to white, bruising purplish-brown slightly, stipe frequently hollow at maturity. Exsiccata dark brown. Habit scattered to subcaespitose. Odor distinctly spicy somewhat like cedar wood. Taste mild. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (6.8–)7.2–8.2(–9.0) × (4.8–)5.0–5.6(–6.0) µm (mean 7.8 × 5.2 µm), Q = 1.42–1.60, Q_{av} = 1.50 (N = 134, 5 basidiomata, four collections), amygdaliform to ellipsoid, ovoid, or broadly ellipsoid, finely verrucose, non-dextrinoid. Basidia 26–40 × 6–8 µm, 4-spored (rarely 2-spored), cylindro-clavate, clamped. Lamella edge sterile. Cystidia not observed. Lamellar trama regular, composed of more or less parallel, cylindrical hyphae, 3–7 µm wide. Pileipellis a cutis; epicutis composed of thin parallel hyphae, 2–7 µm wide with brown pigment in KOH; walls smooth to very finely zebra-stripe encrusted. Lower layer hyphae 8–22 µm wide, with thin walls. No distinct reaction with Melzer's reagent. Hypodermium well developed. Oleiferous hyphae infrequent. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius ohlone* is fairly common under evergreen oaks throughout California and its distribution appears limited to the state. The primary mycorrhizal associates are coast live oak (*Q. agrifolia*) and interior live oak (*Q. wislizenii*).

Fig. 30. *Cortinarius ohlone*
a) DBB41040, an annular zone on the stipe b) DBB42193 (UC 1861349, holotype)

Additional collections examined: **USA. California:** Contra Costa County, San Pablo Dam (37°54′16″N 122°12′55″W), elev. 150 ft, under *Q. agrifolia,* 2 Jan 2008, DBB05460 (Genbank nrITS JQ906760); Santa Cruz County, Santa Cruz, UCSC Campus (36°59′49″N 122°04′02″W), elev. 600 ft, under *Q. agrifolia,* 22 Dec 2010, DBB41040 (Genbank nrITS JQ906758); Riverside County, Cleveland National Forest, off Forest Road 6S05 (33°38′18″N 117°25′21″W), elev. 1300 ft, under *Q. agrifolia, Q. wislizenii,* 12 Feb 2011, DBB42536; Yolo County, Putah Creek State Wildlife Reserve, Franklin Canyon, (38°30′23″N 122°05′51″W), elev. 1000 ft, under *Q. agrifolia, Q. wislizenii,* 6 Jan 2011, R. M. Davis RMD101205 (Genbank nrITS JQ906759);

Discussion — *Cortinarius ohlone* is the most common of several small to medium-sized brownish *Telamonia* species in the oak woods of northern California. The diversity of this group seems to increase in the warmer oak habitats of southern California. A key diagnostic feature for differentiating *C. ohlone* from other similar cortinarii in the same habitat is the slight to pronounced spicy-fragrant odor (reminiscent of cedar wood), which is typical for the members of the /decipiens clade.

Fig. 31. *Cortinarius ohlone* a) DBB42536, older basidiomata b) RMD101205

Fig. 32. *Cortinarius miwok* (UC 1861351, holotype).

Cortinarius miwok Bojantchev, **sp. nov.** Figs 32–34

MycoBank MB 800347

Differs from *C. ohlone* by its coniferous association and montane vernal fruiting habit.

Type: USA. California: El Dorado County, El Dorado National Forest, 0.1 mi. SE of Icehouse Road (38°51′24″N 120°22′32″W), elev. 5100 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 5 Jun 2011, DBB43810 (**Holotype** UC 1861351; Genbank nrITS JQ906753).

Etymology: In honor of the Miwok Native American people — this species can frequently be found in the Sierra Nevada areas that they inhabited.

Pileus 30–70 mm diam., convex to plano-convex, often with a broad flattened umbo; margin involute then straight to flanging; dark to light grey-brown, turning brown at maturity, the extreme margin whitish due to persistent veil remnants; surface fibrillose, smooth to shiny, inconspicuously hygrophanous. Lamellae moderately crowded to subdistant, 6–20 mm broad, clay brown, no bluish tinges, turning rusty brown as the spores mature; edges slightly uneven, pale; attachment sinuate; lamellulae abundant in series of 3–7. Stipe 30–80 mm long, 15–30 mm wide, cylindrical to subclavate, white to light brown beneath the universal veil remnants, bruising purplish-brown, rarely with bluish tinges

Fig. 33. *Cortinarius miwok* a) Basidiospores (UC 1861351, holotype) b) DBB43988

near the top on young basidiomata. Universal Veil white, partially covering the stipe in the lower half, leaving an evanescent annular zone. Cortina white. Context beige to white, bruising purplish-brown slightly, stipe frequently hollow at maturity. Exsiccata dark brown. Habit scattered to subcaespitose. Odor slightly spicy, or moldy if surrounded by wet soil. Taste mild. UV Light no fluorescence detected. Macrochemical reactions 5% KOH negative. Spore deposit rusty brown.

Basidiospores (6.8–)7.2–9.0(–9.3) × (3.8–)4.0–5.0(–5.3) µm (mean 8.2 × 4.5 µm), Q = 1.60–2.14, Q_{av} = 1.82 (N = 177, 7 basidiomata, four collections), amygdaliform to cylindro-amygdaliform, finely verrucose, non-dextrinoid. Basidia 28–38 × 6–9 µm, 4-spored (occasionally 2-spored), cylindro-clavate, clamped. Lamella edge sparsely fertile. Cystidia not observed. Lamellar trama regular, composed of more or less parallel, cylindrical hyphae, 2–8 µm wide. Pileipellis a cutis; upper layer composed of thin parallel hyphae, 2–6 µm wide with olivaceous-brown pigment in KOH; walls smooth to finely zebra-stripe encrusted. Lower layer hyphae 7–20 µm wide, with thin walls. No distinct reaction with Melzer's reagent. Hypodermium well developed. Oleiferous hyphae infrequent. Clamp connections common on all septa.

Habitat and distribution — *Cortinarius miwok* fruits in late spring and early summer in the California mountains during, or soon after, snow melt. It is one of the less common species amongst the vernal fruiting cortinarii. Apparently it occurs in the Pacific Northwest, where it may be uncommon, as there is only one matching sequence in GenBank (FJ039540; also vernally fruiting but incorrectly designated as *C. sertipes* from a collection made at Observatory Hill, Vancouver Island, British Columbia]. In California *C. miwok* occurs in the foothills and higher elevations of the Sierra–Cascade range, where it can frequently be observed near melting snow. The surrounding conifer habitat consists primarily of ponderosa pine (*P. ponderosa*), white fir (*A. concolor),* red fir (*A. magnifica*), lodgepole pine (*P. contorta* var. *latifolia*) and Douglas-fir (*Ps. menziesii*).

Additional collections examined: **USA. California**: El Dorado County, El Dorado National Forest, 0.1 mi. SE of Icehouse Road (38°51′24″N 120°22′32″W), elev. 5100 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 5 Jun 2011, DBB43976 (Genbank nrITS JQ906754); Tuolumne County, Yosemite National Park, off Tioga Pass Rd. (37°50′12″N 119°40′48″W), elev. 7300 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 20 Jun 2009, DBB16312 (Genbank nrITS JQ906751); Sierra County, SF State Sierra Nevada Field campus (39°37′23″N 120°34′48″W), elev. 5700 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 9 Jun 2011, DBB43988 (Genbank nrITS JQ906755); Chapman Creek Campground (39°37′54″N 120°32′37″W), elev. 5900 ft, under *P. ponderosa, A. concolor, A. magnifica, Ps. menziesii,* 22 Jun 2010, DBB33741 (Genbank nrITS JQ906752);

Discussion — *Cortinarius miwok* stands out from the other vernal cortinarii with similar stature in the same surroundings by the more bluish-grey cap and slight purplish discoloration of the context. This species also exhibits the slightly spicy odor typical of the /decipiens clade. Its closest relative in the same habitat is *C. tuolumnensis*, which can be distinguished by its predominantly caespitose habit and longer stems. Microscopically, *C. miwok* can be separated from *C. tuolumnensis* by the smaller spores.

Fig. 34. *Cortinarius miwok* a) DBB16312 b) DBB43976

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Acaulospora endographis (*Glomeromycetes*), a new fungus with a complex spore wall

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Abstract — A new acaulosporoid fungal species characterized by glomerospores with a complex ten-layered wall structure and uniquely ornamented fourth layer (outer wall layer OWL4) found in impacted areas of the Amazon Forest and cultivated areas of the Atlantic Forest in Brazil is described as *Acaulospora endographis*.

Key words — *Glomeromycota*, *Diversisporales*, *Acaulosporaceae*, morphology

Introduction

Several arbuscular mycorrhizal (AM) fungal species with a generally scutellosporoid spore development have been described from tropical areas (Cuenca & Herrera-Peraza 2008; Silva et al. 2008; Oehl et al. 2008, Goto et al. 2009, 2010, 2011, 2012; Tchabi et al. 2009). In contrast, only a few species with an acaulosporoid development have been described during the last 10 years from the tropics [*Ambispora brasiliensis* (Goto et al. 2008), *Acaulospora punctata* (Oehl et al. 2011a), *Ac. sieverdingii* (Oehl et al. 2011b), and *Ac. soloidea* (Vaingankar & Rodrigues 2011)], with the first two described from South America.

An undescribed ornamented acaulosporoid fungus frequently found during diversity studies of arbuscular mycorrhizal fungi in Brazilian biomes is here described and illustrated as *Acaulospora endographis*.

Material & methods

Study area and sites

Soil samples were collected in the Seringal Cachoeira (7°07′–11°08′S 66°30′–74°00′W), located in the municipality of Xapuri, Acre State, north Brazil. Soils were characterized as red-yellow Argissols (Ultisols). The climate in the region is Am' Equatorial (Köppen classification): hot and humid with 24.5–32°C average temperatures with two distinct seasons: dry (300–375 mm precipitation) and rainy (1200–1360 mm). The study areas consisted of an open rainforest dominated by palms, a 20 year-old secondary forest, and a 30 year-old regenerated pasture with *Brachiaria decumbens* Stapf, *Bertholletia excelsa* Bonpl., and *Zea mays* L. The soil contained 4.77–3.44 $mg.dm^{-3}$ organic phosphorus, 0.31–0.21 $cmol_c.kg^{-1}$ potassium, 6.04–3.78 $cmol_c.kg^{-1}$ calcium, 2.88–2.62 $cmol_c.kg^{-1}$ magnesium, 2.62–2.44 $cmol_c.kg^{-1}$ H+Al, and 41–26 $g.kg^{-1}$ organic matter with a pH of 5.60–5.35. Other soil samples, also characterized as red-yellow Argissols (Ultisols), were collected from the Experimental Station of Itapirema (Instituto Agronômico de Pernambuco) in the municipality of Goiana (7°38′20″S 34°57′10″W), Pernambuco State, northeast Brazil, where the climate is Ams' (Köppen classification): tropical rainy with 21.1–31.3°C average annual temperature and 2000 mm average annual rainfall. The soil was sampled during the rainy season (June 2011) in an area cultivated for nine months with cassava (*Manihot esculenta* Crantz); this area was fertilized with NPK and the pH corrected with calcitic limestone at planting time. The soil composition was 3.8 $mg.dm^{-3}$ organic phosphorus, 0.006 $cmol_c.kg^{-1}$ potassium, 0.005 $cmol_c.kg^{-1}$ calcium, 0.003 $cmol_c.kg^{-1}$ magnesium, and 31.0 $g.kg^{-1}$ organic matter with a pH of 5.3.

Morphological analyses

Acaulosporoid spores were extracted from field soils and from trap cultures by wet sieving and sucrose centrifugation (Sieverding 1991). The spores were isolated in a stereomicroscope and mounted in polyvinyl alcohol–lacto–glycerin (PVLG) and PVLG + Melzer's reagent (Brundrett et al. 1994). Slide mounts were dried (3–5 days at 60°C) to clarify oil drops and observed with a Zeiss Axioskop compound microscope. Images of glomerospores were taken using a Canon digital camera.

General terminology follows Goto et al. (2008) and Oehl et al. (2011a), and spore terminology follows Goto & Maia (2006). Holotype and isotype specimens are conserved at the Herbarium of Departamento de Micologia, Universidade Federal de Pernambuco (URM), and other specimens at the Herbarium of Departamento de Botânica, Ecologia e Zoologia, Universidade Federal do Rio Grande do Norte (UFRN).

Arbuscular mycorrhizal cultures

Cultures of the new species were tentatively established in three institutions, two in Brazil and one in Argentina. For culturing the native AM fungal communities 400 g of field soil were placed into 500 ml pots at the Departamento de Ciência do Solo, Universidade Federal Rural do Rio de Janeiro (Seropédica, Rio de Janeiro, BR). *Brachiaria decumbens* was used as bait plant and the culture pots were maintained in the greenhouse. At the Departamento de Micologia, Universidade Federal de Pernambuco (Recife, Pernambuco, BR), cultures were set up using 800 g of field soil collected from Pernambuco into 1000 mL pots. Maize (*Zea mays*), sunflower (*Helianthus annuus* L.), and sorghum (*Sorghum bicolor* (L.) Moench) were used as bait plants and the pots were kept in the greenhouse. At the Microbiology Laboratory, EEA INTA Balcarce, Argentina,

for single species cultures multiple glomerospores were separated from field samples and used as infective propagules using 800 g of field soil collected from Acre into 1000 mL pots, with *Secale cereale* L. as host plant. The cultures were maintained (one year) in the greenhouse. So far, all trials failed to establish a successful symbiosis of the new AMF species either in trap cultures or in the single species cultures.

Taxonomy

Acaulospora endographis B.T. Goto, **sp. nov.** FIGS 1–9

MycoBank MB 564888

Differs from other *Acaulospora* species by its complex ten-layered spore wall structure with spinose ornamentation in the fourth outer wall layer.

TYPE: Brazil, Pernambuco State, Goiana, Experimental Station of Itapirema (Instituto Agronômico de Pernambuco), from red-yellow Argissols under Cassava cultivation (**Holotype**, URM83586; **isotypes,** URM83586a, URM83586b).

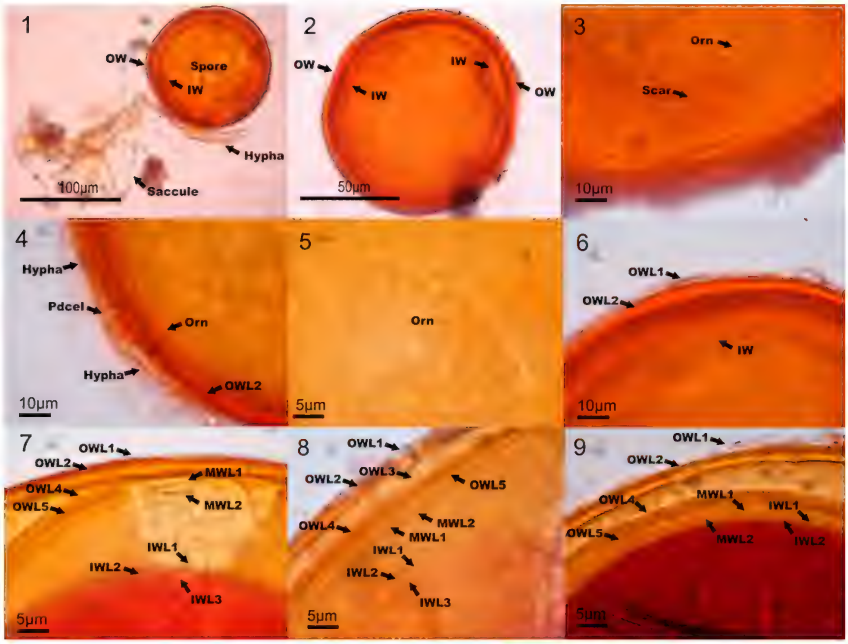
ETYMOLOGY: *endographis* refers to ornamentation similar to pencil imprints in an inner layer of the spore wall.

SPOROCARPS unknown. Acaulosporoid spores form singly in soils upon a short pedicel arising laterally on the tapering hyphal neck of a sporiferous saccule. The glomerospore forms three spore walls — outer wall (OW), middle wall (MW), and inner wall (IW) — with ten layers total.

SPORIFEROUS SACCULE hyaline to white, globose to subglobose and formed terminally on a hypha, generally absent and detached from the mature spore while the pedicel or collar often persists on the glomerospore. The saccule wall is generally bi-layered with a rapidly degrading evanescent hyaline to light yellow outer layer (0.5–1.5(–2.0) µm) and semi-persistent hyaline to subhyaline inner layer 0.8–1.5(–2.2) µm.

GLOMEROSPORES form singly on a short hyphal pedicel. The spores are globose (74–128 µm diam.) to subglobose to oval (68–110 × 69–100(–180)), yellow brown to orange brown in water (generally dark orange to dark orange brown in PVLG-based mountants). The spore wall is complex with ten layers distributed in the three walls (OW, MW, IW).

OUTER WALL consists of five layers, with the outermost layer (OWL1) evanescent, 0.5–1.5 µm thick, hyaline to sub-hyaline, and often difficult to detect, as it tightly adheres to OWL2. OWL2 is laminated, 2.2–5.4 µm thick, and yellow to orange brown. OWL3 is thin (< 0.5 µm), hyaline, and tightly attached to OWL2, making it difficult to detect. In older spores, the outer wall layer might degrade completely, but usually some fragments remain on the OWL2 surface. A fourth layer (OWL4) is 2.5–4.5 µm thick, yellow to yellow brown and ornamented with spines, generally 0.5–1.0(–1.5) µm wide and 1.5–2.0(–2.5) µm high. OWL5 tightly adheres to OWL4 and is flexible, thin (< 1.0 µm), hyaline and difficult to see. No Melzer's reaction is evident in any outer wall layer.



Figs 1–9. *Acaulospora endographis*. **1.** Young spores with hyaline to sub-hyaline sporiferous saccule attached. **2.** Intact glomerospores with distinctive outer wall (ow) and inner wall (iw). **3.** Cicatrix globose to subglobose commonly observed in healthy spores; note the ornamentation (Orn) in a planar view. **4.** Insertion point of hypha in spore with a short pedicel (Pdcel). In this case the pedicel wall is continuous with owl1. **5.** Ornamentation in a planar view. **6.** Spore wall with distinctive outer wall (OW) and inner wall (IW). 7–9. Spore wall structure with OW, middle wall (MW) and IW. Note the outer wall with five layers (owl1,2,3,4,5) and ornamentation in owl4. Inner wall presents a beaded layer (IWL1) and a strong Melzer's reaction.

Middle wall is bi-layered and 2.0–4.0 µm thick. MWL1 is semi-rigid to flexible, 1.0–1.5 µm thick, hyaline, and tightly adheres to MWL2, also semi-rigid to flexible, 1.0–2.0 µm, and hyaline. The bi-layered wall component appears laminated in some spores. No Melzer's reaction is evident in either middle wall layer.

Inner wall is three-layered, with all three layers hyaline. The outer layer IWL1 is 0.5–1.0 µm and easy to detect, ornamented with granular (beaded) projections, and tightly adherent to IWL2 which is amorphous and generally 1.5–3.5 µm thick. IWL3 adheres to IWL2 and is thin (0.5–1.0 µm thick) and difficult to see. The Melzer's reaction is dark red purple in IWL2.

Pedicel short, 2–4 µm long, and 4–6(–8) µm wide at the glomerospore base. The wall layer of the pedicel is 3–4 µm thick and sub-hyaline to light yellow. The pedicel wall layer is always continuous with the outer spore wall

layer (OWL1), and the OWL2 sometimes forms a collar on the glomerospore (2.5–4 µm diam.)..

Germination was not observed. The germination structure is believed to resemble those observed in other *Acaulospora* species (see Spain 1992).

Arbuscular mycorrhiza formation is unknown.

Distribution — So far, known only from Brazil in soils from disturbed areas of the Amazon Forest (North Brazil) and in agricultural systems from the Atlantic rainforest (Northeast Brazil).

Additional material examined: **BRAZIL, Acre State,** Xapuri, Seringal Cachoeira, from red-yellow Argissols (UFRN1742 [permanent slides with PVLG]).

Discussion

The low number of glomerospores (2–6) in 100g of the field soil samples from both sites explains the difficulty in obtaining trap and pure cultures of the new fungus during this study.

Its complex distinct spore wall and ornamentation easily separate *Acaulospora endographis* from other acaulosporoid species. A similar ornamentation is found only in *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid., but the entrophosporoid spore development and two-walled structure easily distinguish *E. infrequens* from *A. endographis*. Furthermore, the evanescent or semi-persistent layers covering the ornamentation in *E. infrequens* (Sieverding & Oehl 2006, Oehl et al. 2011c) is quite distinct from the permanent rigid laminated layer covering the ornamentation in *A. endographis.*

The complex 10-layered spore wall structure with one spiny ornamented layer (OWL4) easily distinguishes *A. endographis* from all other ornamented *Acaulospora* species.

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Basidiospore ultrastructure of some *Dacrymycetales* from Mexico

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Abstract — An ultrastructural study was made of basidiospores from nine species of *Dacrymycetales*: *Calocera macrospora*, *C. viscosa*, *Dacrymyces dictyosporus*, *D. chrysospermus*, *Dacryopinax elegans*, *Da. spathularia*, *Da. lowyi*, *Guepiniopsis alpina*, and *G. buccina*. Spore walls and septa analyzed with transmission electron microscopy revealed the absence or presence of discrete lateral wall layers as well as septal pores that appear diagnostic at the species level.

Key words —jelly fungi, morphology, systematics, taxonomic novelty

Introduction

Septum ultrastructure has been shown to serve as a highly significant phylogenetic marker of the order *Dacrymycetales* (Grand & Moore 1971, Khan & Talbot 1976, Moore 1978, 1994; Khan & Kimbrough 1982, Berbee & Wells 1988, 1989; Boehm & McLaughlin 1989, Lu & McLaughlin 1991, Wells 1994). Even when teleomorphic characters are lacking, the septal pore ultrastructure together with some other character (e.g. pigmentation) facilitates placement of certain taxa within the order (Kirschner & Yang 2005). It is, however, not clear whether there are other ultrastructural characters that permit differentiation at the specific, generic, or family level within the order. Here we describe and compare the ultrastructure of basidiospore walls and septa in several taxa of *Dacrymycetales* from Mexico.

Materials & methods

Seventy-five specimens representing nine dacrymycete species from México were collected. Species represented were *Calocera macrospora, C. viscosa*, *Dacrymyces*

chrysospermus, *D. dictyosporus*, *Dacryopinax elegans*, *Da. lowyi*, *Da. spathularia*, *Guepiniopsis alpina*, and *G. buccina*. The specimens were examined using standard mycological techniques (Martin 1952, Lowy 1971, Cifuentes et al. 1986, Sierra 1992, 1995, 2000; Sierra & Cifuentes 1993) and deposited in FCME and TLXM herbaria (Holmgren & Holmgren 1990).

Ten specimens with a large number of spores were selected for processing with transmission electron microscopy (TEM). Small (2 mm^3) basidiome pieces were rehydrated for 1 day in distilled water. Samples were fixed with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4 for 2 hours. A post-fixation with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer was carried out for 2 hours. The samples were dehydrated via a graded ethanol series (70–100%), prepared with a mixture of propylene oxide and epoxy resin (in 2:1, 1:1, and 1:2 proportions) for 24 hours, and embedded in pure epoxy resin. The block face was then shaped to a 0.1 mm^2 trapezoid. Sections were cut with glass cleavers in a Sorvall Ultramicrotome MT2. No serial-sections were made due to the scarcity of herbarium materials in good condition. Sections were placed on copper grids and stained in uranyl acetate (10 minutes) followed by lead citrate (5 minutes). The grids were examined with a Zeiss EM-10 TEM.

Species & collections

Calocera macrospora Brasf., Lloydia 1: 156. 1938. Figs 1a,b

Specimen examined: **MEXICO, Tlaxcala**, Mpio. de Tlaxco, Parque recreativo El Rodeo, 19°39′54″N 98°08′17″W, alt. 2900 m, on rotten wood, 14 VIII 1992, Pérez-Ramírez 1604 (FCME 4856).

Calocera viscosa (Pers.) Fr., Syst. Mycol. 1: 486. 1821. Figs 1c,d

Specimen examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Desviación al Balneario Erendira, km 5.5 carr. San Pedro Jacuaro–Los Azufres, 19°45′36″N 100°41′21″W, alt. 2600 m, on soil, 4 IX 1987, Ojeda y Villegas-Ríos 952 (FCME 14002).

Dacrymyces chrysospermus Berk. & M.A. Curtis, Grevillea 2: 20. 1873. Figs 3a,b

Specimen examined: **MEXICO, Guerrero**, Mpio. de Chilpancingo de los Bravo, Cañada de Agua Fría, 17°32′30″N 99°41′30″W, alt. 2500 m, on rotten wood, 8 VII 1984, López L. s/n (FCME 13012).

Dacrymyces dictyosporus G.W. Martin, Mycologia 50: 939. 1959. Figs. 2

Specimen examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Desviación al Balneario Erendira, km 5.5 carr. San Pedro Jacuaro-Los Azufres, 19°45′36″N 100°41′21″W, alt. 2600 m, on rotten wood, 4 IX 1987, Molina y Pérez-Ramírez 805 (FCME 14137).

Dacryopinax elegans (Berk. & M.A. Curtis) G.W. Martin, Lloydia 11: 116. 1948. Fig. 3c

Specimen examined: **MEXICO, Tabasco**, Mpio. de Teapa, Centro Regional Universitario del Sureste (CRUSE) Puyacatengo, 17°31′39″N 92°55′51″W, alt. 80 m, on rotten wood, 20 X 1991, Sierra 46 (FCME 4523).

Dacryopinax lowyi S. Sierra & Cifuentes, Mycotaxon 92: 244. 2005. Figs 3f,g

Specimens examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Laguna Larga, zona de protección forestal Los Azufres, 19°48′05″N 100°41′02″W, alt. 2800 m, on

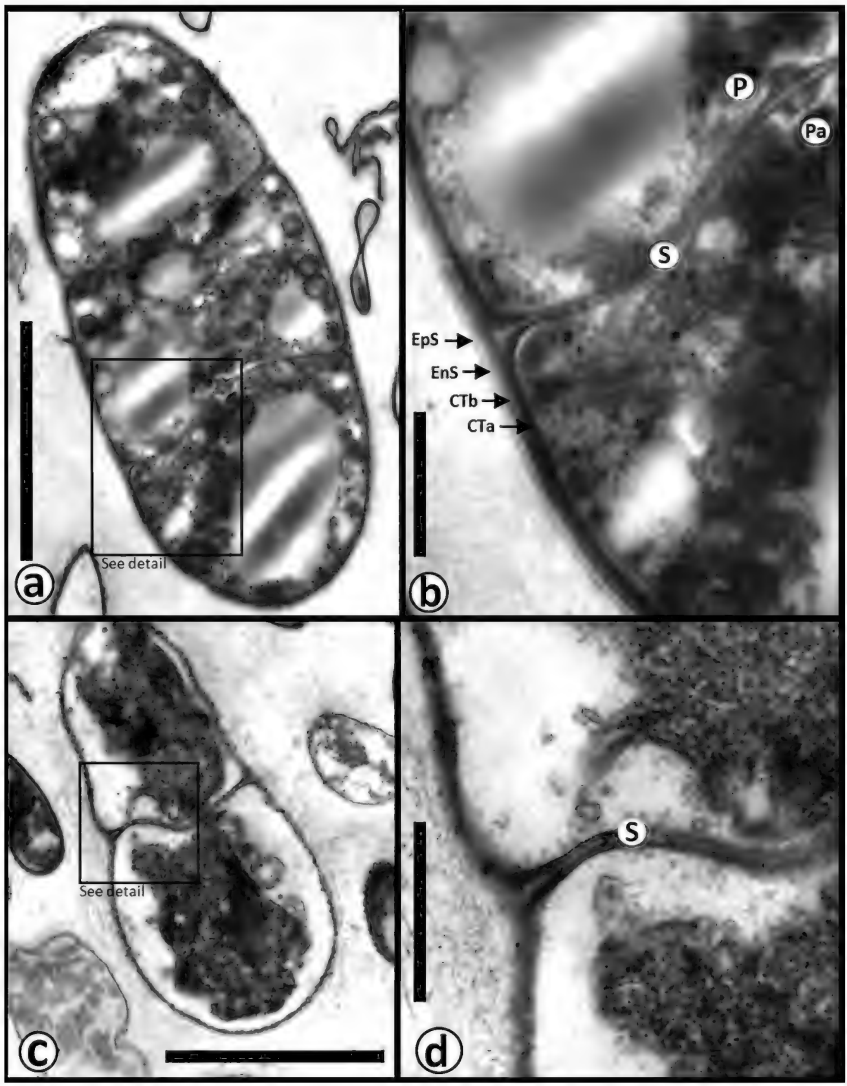

FIGURE 1. ***Calocera macrospora***: **a**. Biseptate basidiospore. **b**. Detail of spore wall and septum [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium= EpS, septum = S, pore = P, pore aperture = Pa]. ***Calocera viscosa***: **c**. Uniseptate basidiospore. **d**. Detail of septal wall [S]. Bars: a, c = 5 μm; b, d = 1 μm.

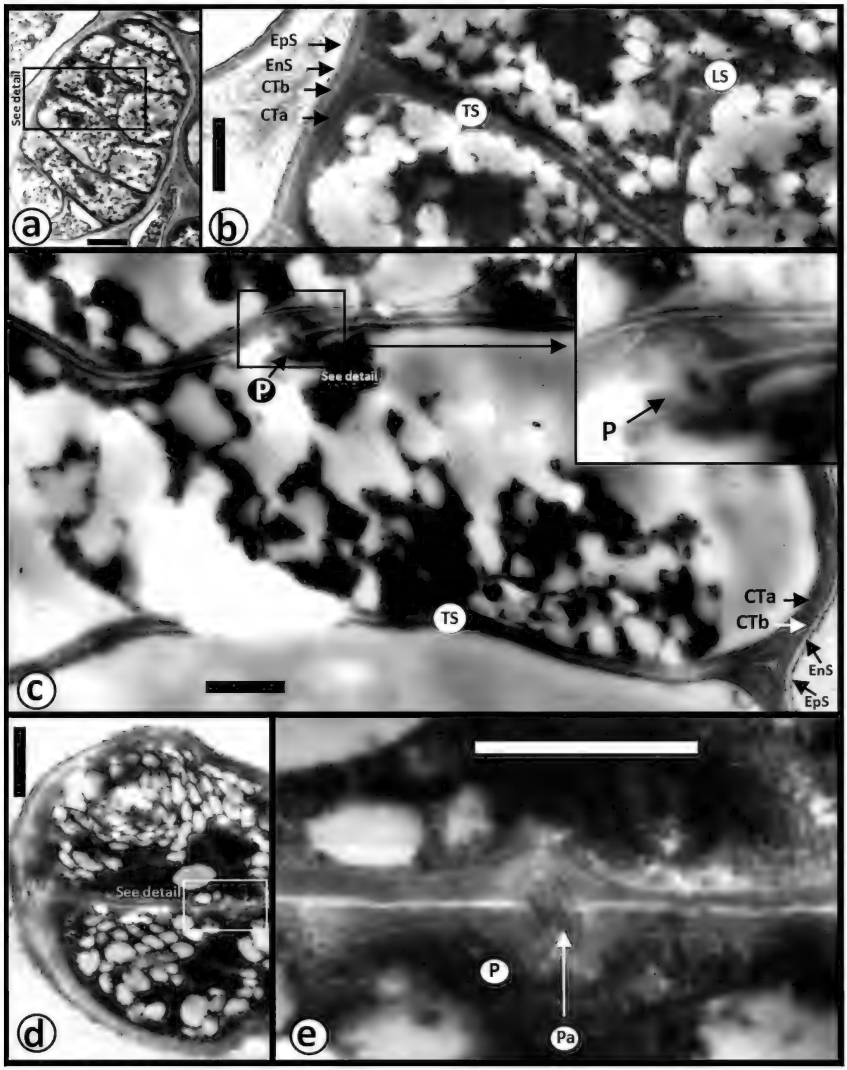

FIGURE 2. *Dacrymyces dictyosporus*: **a**. Multiseptate basidiospore (transverse and longitudinal septa). **b**. Detail of spore wall and septum [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium = EpS, transverse septum = TS, longitudinal septum = LS]. **c**. Detail of a multiseptate basidiospore; only transverse septum [TS] and spore wall with 4 layers [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium = EpS, pore = P]. **d**. Uniseptate basidiospore. **e**. Detail of septal wall [pore = P, pore aperture = Pa]. Bars: a = 5 µm; b–d = 2 µm; e = 1 µm.

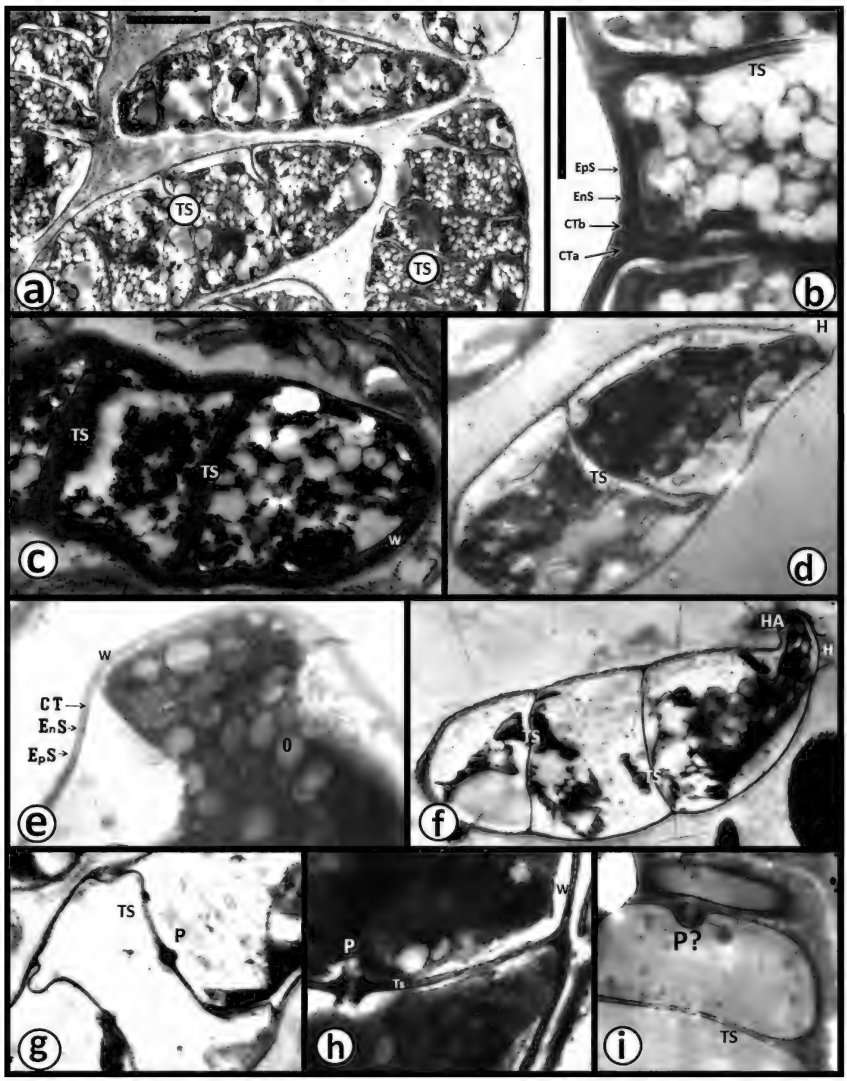

FIGURE 3. ***Dacrymyces chrysospermus***: **a**. Multiseptate basidiospore with transverse septa [TS] only. **b**. Detail of spore wall and septa. ***Dacryopinax elegans***: **c**. Spore walls [W] and transverse septa [TS]. ***Dacryopinax spathularia***: **d**. Uniseptate basidiospore with hilar appendix [HA]. **e**. Detail of spore wall [W]. ***Dacryopinax lowyi***: **f**. Biseptate basidiospore with hilar appendix [HA]. **g**. Detail of transverse septum [TS] and pore [P]. ***Guepiniopsis buccina***: **h**. Detail of spore wall [W], transverse septum [S], organelle, and pore [P]. ***Guepiniopsis alpina***: **i**. Detail of transverse septum [TS] and pore [P?]. Bar: a, b = 5 μm.

rotten wood, 3 VII 1987, Núñez Mariel s/n (FCME 13863); **Estado de México**, Mpio. de Amecameca, Km 14 carr. Amecameca–Tlamacas, 19°05′14″N 98°40′43″W, alt. 3300 m, on rotten wood, 15 VII 1993, Sierra 165 (FCME 20406).

Dacryopinax spathularia (Schwein.) G.W. Martin, Lloydia 11: 116. 1948. Figs 3d,e

Specimen examined: **MEXICO, Nayarit**, Mpio. de Tepic, La Capilla, Reserva Ecológica del Cerro San Juan, 21°28′11″N 105°00′31″W, alt. 1371 m, on rotten wood, 29 VIII 1991, Rodríguez Castañeda y Pérez-Ramírez 1467 (FCME 3903).

Guepiniopsis alpina (Tracy & Earle) Brasf., Amer. Midl. Nat. 20: 225. 1938. Fig. 3i

Specimen examined: **MEXICO, Hidalgo**, Mpio. de Tenango de Doria, Ejido Muridores a 2 km de Apulco vía Agua Blanca, 20°20′05″N 98°20′52″W, alt. 2200 m, on rotten wood, 3 IX 1980, Cifuentes 827 (FCME 10620).

Guepiniopsis buccina (Pers.) L.L. Kenn., Mycologia 50(6): 888. 1959. Fig. 3h

Specimen examined: **MEXICO, Hidalgo**, Mpio. de Calnali, a 8 km de Ahuacatlan, 20°49′N 99°03′W, alt. 1300 m, on rotten wood, 8 VII 1980, Cifuentes 480 (FCME 10159).

Results

Calocera macrospora and *C. viscosa* have uniformly thick walls. In *C. viscosa* the wall layers are not defined, but they are in septa, while in *C. macrospora* the layers are clearly defined. A septal pore-like structure was observed only in *C. macrospora*.

Dacrymyces dictyosporus has both transverse and longitudinal septa, while only longitudinal septa are present in *D. chrysospermus*. The spore walls of these two species are thick, with four well-differentiated layers, and 3-layered septa are visible in each one. The *D. dictyosporus* septa have a wall with a central swelling (pore-like structure) that is not observed in *D. chrysospermus*.

Spore walls are not uniformly thick within *Dacryopinax*. In *Da. elegans* spore walls are thick but with undefined layers; two septal wall layers can be seen, but no septal pores are distinguished. Spore walls in *Da. spathularia* are thick (but not to the same degree as in *Da. elegans*) and the walls are 3-layered. In *Da. lowyi* (Sierra & Cifuentes 2005), a hilar appendix, broken hilum wall, and septal pores with a dolipore-like structure can be discerned.

Spore wall layers are visible in *Guepiniopsis buccina* and there is a pore-like structure in the septum. In *G. alpina* there is a swollen part in the transverse septal wall that is not dolipore-like. The results are summarized in Table 1.

Discussion

All our conclusions are based on well-fixed material, but the material may have been damaged during drying before fixing, and we could not make serial sections. Nonetheless, the presence or absence of spore septal pores

Table 1. Presence or absence of spore wall layers and septa with dolipore.

Species	Lateral spore wall a distinct layer	Spore septa with dolipore
Calocera macrospora	+	+
C. viscosa	–	–
Dacrymyces chrysospermus	+	–
D. dictyosporus	+	+
Dacryopinax elegans	–	–
Da. lowyi	–	+
Da. spathularia	+	–
Guepiniopsis alpina	–	– (?)
G. buccina	+	+

and distinguishable lateral wall layers appear species specific in *Calocera*, *Dacrymyces*, *Dacryopinax*, and *Guepiniopsis*. The differences observed are unlikely to be based on fixation artifacts. We could not distinguish lateral wall layers in the well-preserved spores of either *Da. elegans* or *C. macrospora,* so that the absence of distinguishable lateral wall layers in the less well preserved spores of *C. viscosa* is, therefore, not likely to result from poor fixation. On the other hand, the septal swelling seen in *G. alpina* might well be an artifact that requires further study for verification. Pore-like structures that can be detected in even poorly fixed material can easily be overlooked in single sections. In *Basidiomycota*, the absence of septal pores has been indicated as an important ultrastructural marker at the family and ordinal levels for of smut fungi (e.g., *Microbotryaceae*, *Georgefischeriales, Ustilaginales;* Bauer et al. 1997). Keller (1986, 1992, 1997), Keller & Job (1992), and Garnica et al. (2007) have all studied spore walls, yet none of them have commented on pores in the spore septa.

The morphology and DNA analysis of taxa within the *Dacrymycetales* does not appear to be consistent (Shirouzu et al. 2012). Perhaps some of the ultrastructural differences described here might be useful for characterizing taxa in a new classification more congruent with the DNA analysis. The first step might be in producing good serial sections for a better morphological reconstruction of septal pores in spores.

Acknowledgments

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Studies of two Corner types (*Marasmius nigroimplicatus* and *M. subrigidichorda*) and new *Gymnopus* combinations

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Abstract — Type specimens of *Marasmius nigroimplicatus* and *M. subrigidichorda* were studied. Based on morphological studies, both species belong to *Gymnopus*, sect. *Androsacei*. Color photographs of dried basidiomata and microscopic characters accompany the detailed descriptions. New combinations are proposed for these two taxa. Nine related marasmioid species are transferred to *Gymnopus*.

Key words — *Agaricales, Basidiomycota, Omphalotaceae,* taxonomy

Introduction

Corner (1996) described 99 new white-spored agaric species from Malesian region, of which 87 were placed in the genus *Marasmius*. However, he used a very broad generic concept of *Marasmius* that includes several genera (*Gloiocephala, Gymnopus, Marasmiellus, Rhodocollybia,* etc.) which have mostly been accepted as good genera and whose concepts have also been supported by phylogenetic analyses (e.g., Wilson & Desjardin 2005, Tan et al. 2009, Antonín & Noordeloos 2010). Consequently, most of the *Marasmius* species described by Corner (1996) should be transferred into the new genera.

During the process of describing the new species, *Gymnopus fuscotramus* Mešić et al. (Mešić et al. 2011), we studied types of two similar species described by Corner (1996), *Marasmius nigroimplicatus* and *M. subrigidichorda*, known only from their type localities in Singapore. In this paper we give detailed species descriptions, as well as color photographs of their dried basidiomata and microscopic characters. The two species are characterized by small marasmioid basidiomata, thin insititious dark colored stipes, well-developed black rhizomorphs, dextrinoid trama of stipe, and non-hymeniform pileipellis. According to these characters, neither species belongs to *Marasmius* in the modern sense, but to *Gymnopus,* sect. *Androsacei* (Kühner) Antonín & Noordel.

(Wilson & Desjardin 2005, Noordeloos & Antonín 2008, Antonín & Noordeloos 2010). Therefore, we propose new combinations in *Gymnopus* (Pers.) Roussel for the two studied species, as well as for nine related marasmioid species in sect. *Androsacei* based on their morphological characters.

Materials & methods

The descriptions of *Gymnopus nigroimplicatus* and *G. subrigidichorda* are completely based on the type collections deposited at the Royal Botanic Garden Edinburgh (E). The following abbreviations are used in macroscopic description: L for number of lamellae and l for number of lamellulae between two lamellae. Microscopic features were observed with a light microscope (brightfield and phase contrast (PhC)) under magnification up to 1500× and photographed with a digital camera. Descriptions and images of microscopic characters were made from rehydrated dried specimens mounted in 2.5% potassium hydroxide (KOH) solution. Amyloidity and dextrinoidity were tested in Melzer's reagent (Erb & Matheis 1983). Basidiospore measurements were made from mounts of lamellae and based on calibrated digital images. For *G. nigroimplicatus,* three mature basidiomata were chosen and 20 basidiospores were measured in total (spores were scarce), while for *G. subrigidichorda,* two mature basidiomata were chosen and 50 randomly selected basidiospores were measured in total. Spore measurements (length, width) include apiculus and are given as: (min.) stat. min. – av. – stat. max. (max), where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic average minus 2× standard deviation), "av." = arithmetic average, "stat. max." = statistical maximum (arithmetic average plus 2× standard deviation), "max." = maximum (highest measured value). Standard deviation (SD) of spore length and width is also given. The length/width ratio of spores is given as the "Q" value (min. – av. – max.). Comparison of *G. nigroimplicatus* and *G. subrigidichorda* with similar species is based on descriptions and illustrations in the following literature: Petch 1948, Singer 1965, 1969, 1976, 1989, Pegler 1986, Desjardin 1987, Corner 1996, Antonín 2007, Mešić et al. 2011.

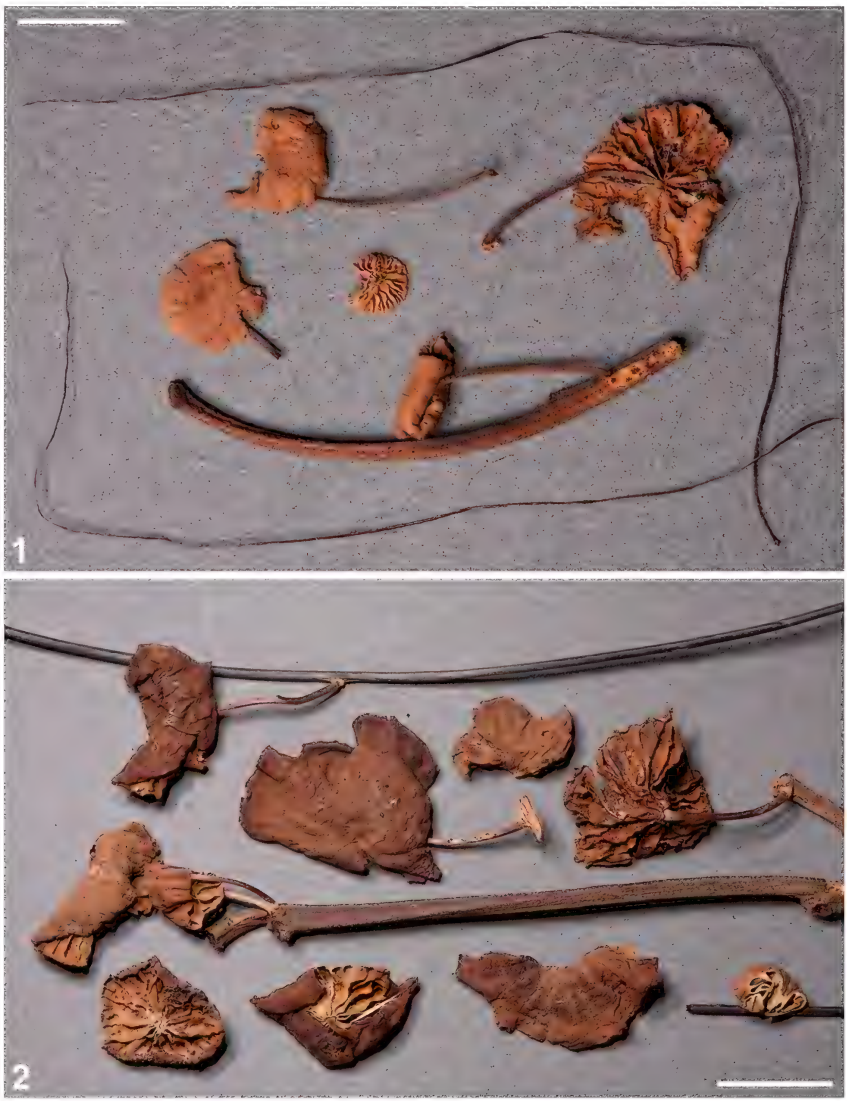
Taxonomy

Type studies

Gymnopus nigroimplicatus (Corner) Mešić & Tkalčec, **comb. nov.** Plates 1, 3–7
MycoBank MB 564875

≡ *Marasmius nigroimplicatus* Corner, Beih. Nova Hedwigia 111: 75. 1996. Type: SINGAPORE: Botanic Garden, 14 Aug 1940, leg. E. J. H. Corner, (E 206719, holotype).

The following description is compiled from the dried holotype collection, which consists of approximately 12 basidiomata, partially fragmented or badly preserved, accompanied with abundant rhizomorphs. Basidiomata and rhizomorphs arising from dead leaves. Basidiomata are not connected with rhizomorphs. Pileus 3–8.5 mm broad, convex or conico-convex at first, later applanate, slightly striate-sulcate, matt, homogeneous brown. Lamellae



Plates 1–2. Dried basidiomata (holotypes).
1. *Marasmius nigroimplicatus*. 2. *Marasmius subrigidichorda*. Bars: 1 = 5 mm; 2 = 10 mm.

adnate or attached to a pseudocollarium, distant, L = 10–14, l = 1–3, ≤1 mm broad, sometimes furcate, brown (concolorous with pileus), with an entire, concolorous edge. Stipe 7–23 × 0.15–0.40 mm, subcylindrical, dark brown, surface densely minutely dark brown pubescent, insititious. Context brown.

Rhizomorphs filiform, mostly gradually tapering towards the apex, ≤70 mm long, 0.05–0.20 mm thick near the base, rarely branched, mostly minutely pubescent (especially in the lower half), dark brown to black.

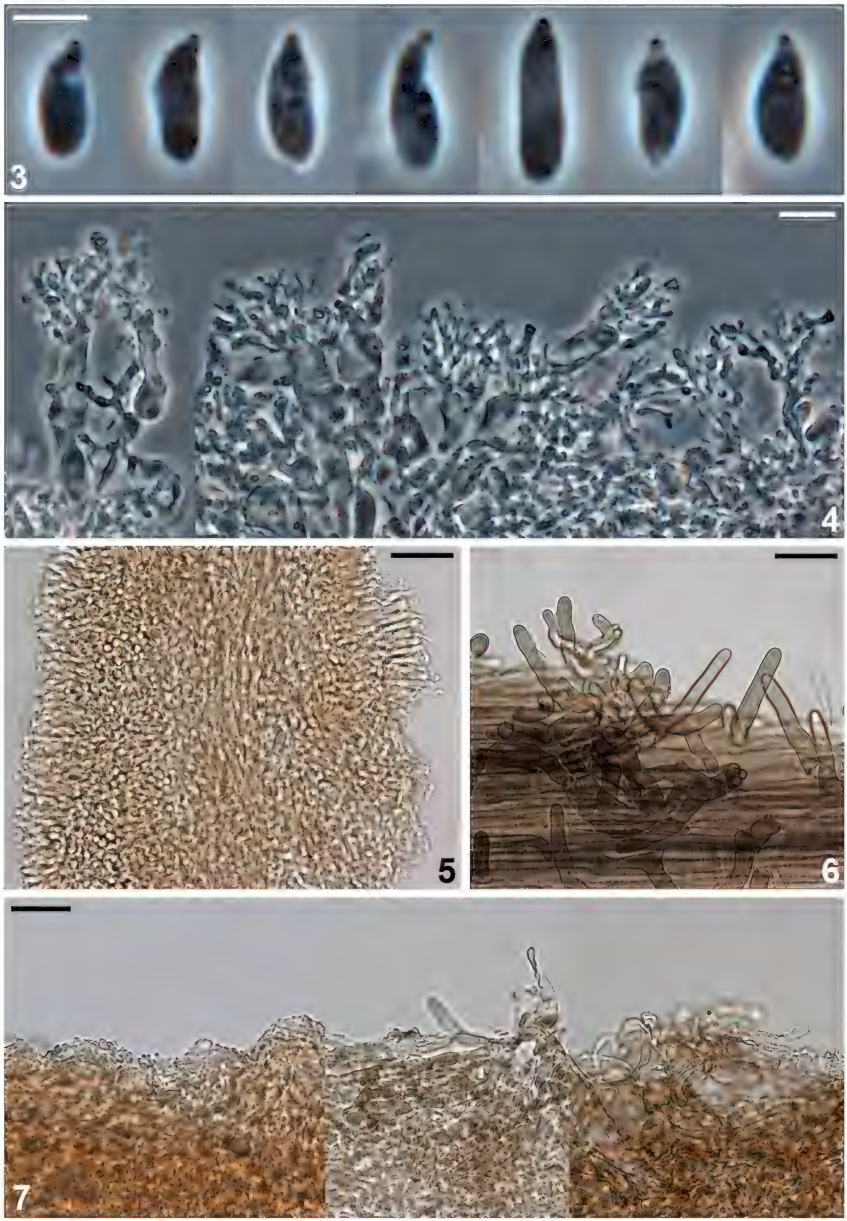
Basidiospores [20/3/1] (7.1–)6.8–*8.7*–10.5(–11.0) × (2.3–)2.3–*3.0*–3.7 (–3.6) µm, SD = 0.93 × 0.36, Q = 2.35–*2.95*–3.87, cylindrical to bacilliform, mostly with conical base and rounded apex in frontal view, (sub)amygdaliform or lacrymoid in side view, smooth, hyaline, thin-walled, non-amyloid, non-dextrinoid. Basidia 20–25 × 6.5–7 µm, clavate, 4-spored, thin-walled, pale brownish to pale brown, light brown in mass. Basidioles mostly fusoid, but also subcylindrical, narrowly utriform or narrowly clavate, often mucronate to rostrate (and then sometimes with subcapitate apex). Lamellar edge sterile. Cheilocystidia abundant, 21–50 × 8–27 µm, coralloid, with clavate to irregularly shaped body (10–30 × 3–9 µm) and mostly abundant, strongly branched projections, thin-walled, hyaline to pale yellowish brown. Pleurocystidia absent. Hymenophoral trama rather irregular, composed of pale brownish to pale brown (light brown in mass), often branched, 1–6 µm broad, thin-walled hyphae. Pileipellis an irregular cutis, hyphae 2–10 µm broad, thin-walled, smooth to diverticulate, terminal elements sometimes subcoralloid, subhyaline to brown, pigment often minutely to coarsely encrusted. Stipitipellis a cutis of parallel, cylindrical, 3–10 µm broad, brown-gray hyphae with encrusted pigment. Caulocystidia abundant, formed as lateral or terminal elevations of stipitipellis cells, 5–93 × 4–12 µm, cylindrical, subcylindrical, less frequently somewhat flexuose, rarely branched, always with obtuse apex, thick-walled (1.8–5 µm thick), brown-gray, pigment parietal. Stipe trama composed of (sub-)hyaline, parallel, thin- to thick-walled (≤4 µm thick), 2–9 µm broad hyphae. Clamp connections present in all tissues.

Chemical reactions — All parts of basidioma non-amyloid and non-dextrinoid except hyphae in cortical layer of stipe and caulocystidia, which are strongly dextrinoid.

Habitat — On dead leaves in botanic garden.

Distribution — Known only from the type locality in Singapore, Asia.

Remarks — *Gymnopus nigroimplicatus* is characterized by purplish brown to cinnamon brown colors in pileus, lamellae, and stipe; slightly striate-sulcate pileus; lamellae adnate or attached to a pseudocollarium; an insititious, dark brown, and minutely pubescent stipe; well-developed black rhizomorphs; basidiomata growing only on dead leaves (not arising from rhizomorphs); non-hymeniform pileipellis (irregular cutis); subcylindrical, cylindrical or lacrymoid spores; presence of clamp connections in all tissues; coralloid cheilocystidia; abundant thick-walled caulocystidia with obtuse apex; and dextrinoid hyphae in cortex of stipe. We consider it a good species.



Plates 3–7. *Marasmius nigroimplicatus*. 3. Spores (PhC). 4. Cheilocystidia (PhC). 5. Hymenophoral trama. 6. Caulocystidia. 7. Pileipellis. Bars: 3 = 5 µm; 4 = 10 µm; 5–7 = 30 µm.

Among other species in sect. *Androsacei* with clamp connections, black rhizomorphs, non-glabrous stipe, and colored (not white or cream) pileus and lamellae, *G. bactrosporus* has a smaller pileus (2.5–3.5 mm broad), whitish to light sordid gray lamellae, longer spores (10–12 × 2.7–3.7 µm), well-developed pleurocystidia, and different cheilocystidia. *Gymnopus brevistipitatus* has a smaller pileus (2–4 mm broad), shorter stipe (2–3 mm long), fewer lamellae (L = 7–9), and smaller caulocystidia (20–40 µm long) with apical projections or coralloid. *Gymnopus campinaranae* has a smaller pileus (2–3 mm broad), thinner stipe (≤0.1 mm wide), and larger spores (9–13 × 3.2–4.5 µm). *Gymnopus fuscotramus* has free lamellae, glabrous and thicker rhizomorphs (≤0.5 mm wide), less elongated spores, and caulocystidia with thinner walls [0.5–2(–2.5) µm thick]. *Gymnopus rhizomorphicola* has a shorter (±3 mm long) eccentric curved stipe, fewer lamellae (L = 7–8), and basidiomata arising from rhizomorphs. *Gymnopus rigidichorda* has mature creamy white lamellae, thicker (0.4–0.6 mm wide) glabrous rhizomorphs, and differently shaped non-coralloid cheilocystidia. *Gymnopus subrigidichorda* has an umbilicate pileus, broader lamellae, longer and thicker rhizomorphs, broader spores (4–5 µm wide), and rare clamp connections (seen only in stipe trama). *Gymnopus thiersii* has rare or absent rhizomorphs, crowded lamellae, and lacks cheilocystidia.

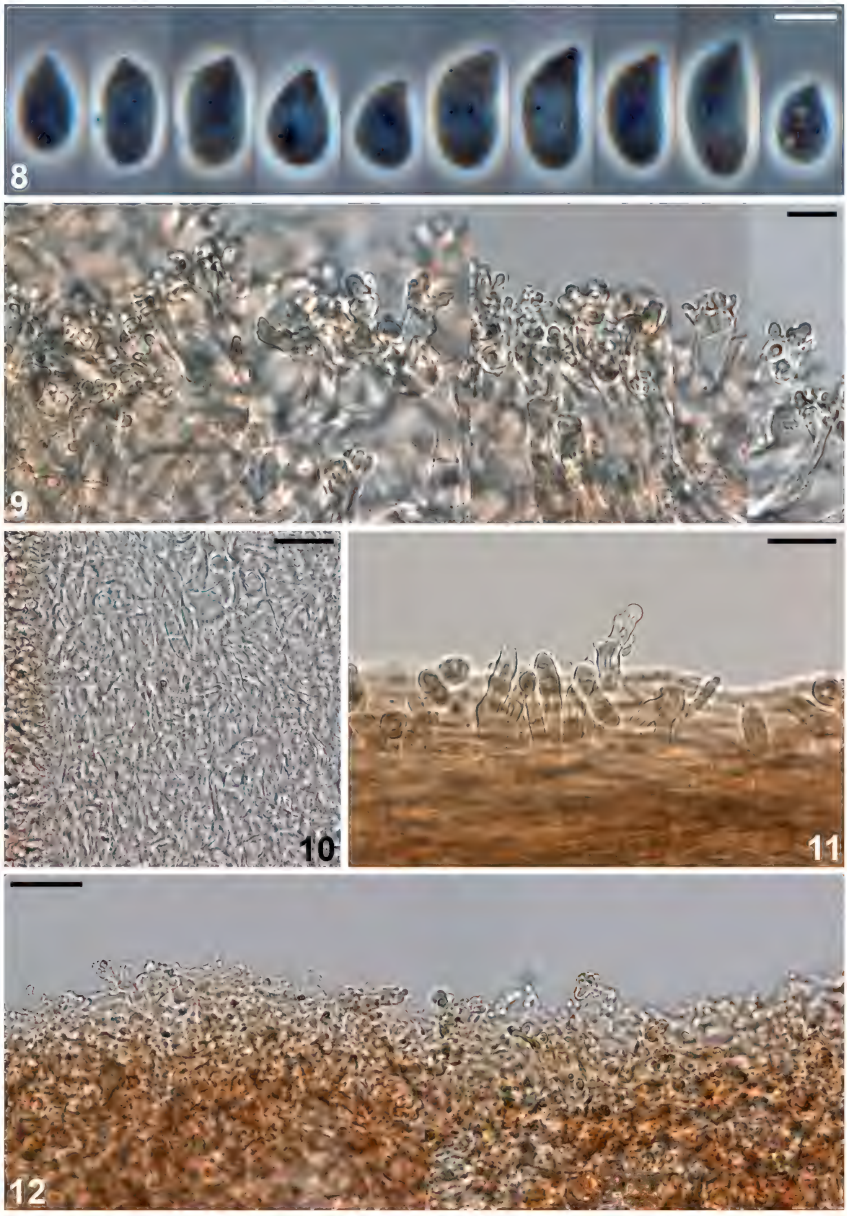
Gymnopus subrigidichorda (Corner) Tkalčec & Mešić, **comb. nov.** Plates 2, 8–12

MycoBank MB 564876

≡ *Marasmius subrigidichorda* Corner, Beih. Nova Hedwigia 111: 99, 1996. Type: SINGAPORE: Bukit Timah, 11 Nov 1929, leg. E. J. H. Corner, (E 206861, holotype).

The following description is compiled from the dried holotype collection, which consists of 20 basidiomata in good condition accompanied with abundant rhizomorphs. Basidiomata arising from 2-8 mm wide twigs as well as from rhizomorphs. Pileus 6.5–19 mm broad, convex to plano-convex with an umbilicate center, sometimes with a small papilla in the central depression, striate-sulcate up to the center, matt, homogeneous brown to dark brown. Lamellae attached to a pseudocollarium, distant, L = 12–14, l = 1–3, ≤2.5 mm broad, often intervenose, yellowish brown to brown, with an entire, concolorous edge. Stipe 4–17 × 0.6–0.9 mm, subcylindrical (slightly broadened at the base and the apex), brown to dark brown, surface moderately to densely minutely brown pubescent (under a hand-lens), sub-insititious with small pale brown fibrils at the base. Context pale brown. Rhizomorphs robust, ≤410 mm long and ≤0.9 mm thick, occasionally branched, glabrous, shiny, stiff, black with a pale brown apex, medulla pale brown.

Basidiospores [50/2/1] (6.2–)6.4–*8.3*–10.2(–10.2) × (4.0–)4.0–*4.5*–5.1(–5.1) µm, SD = 0.95 × 0.27, Q = 1.43–*1.82*–2.18, ellipsoid to elongate (sometimes cylindrical), mostly with conical base and rounded apex in frontal view, mostly



Plates 8–12. *Marasmius subrigidichorda*. 8. Spores (PhC). 9. Cheilocystidia. 10. Hymenophoral trama. 11. Caulocystidia. 12. Pileipellis. Bars: 8 = 5 µm; 9 = 10 µm; 10–12 = 30 µm.

(sub)amygdaliform or lacrymoid in side view, smooth, hyaline, thin-walled, non-amyloid, non-dextrinoid. Basidia 26–38 × 6.5–10 µm, clavate, 4-spored, thin-walled, subhyaline to pale brown, light brown in mass, pigment intracellular and granular. Basidioles narrowly clavate, cylindrical or fusoid, sometimes mucronate to rostrate. Lamellar edge heterogeneous. Cheilocystidia scattered to locally abundant, 13–32 × 7.5–20 µm, ± clavate with irregular apical projections of different types (nodose, digitate or branched), thin-walled, subhyaline to pale brown in KOH. Pleurocystidia absent. Hymenophoral trama irregular, composed of hyaline, sometimes slightly gelatinized, often branched hyphae, 3–14 µm broad, thin- to moderately thick-walled (≤0.8 µm thick). Pileipellis an irregular cutis of mostly nodose to diverticulate ± repent hyphae (3–10 µm broad) and abundant ± erect, lobed to irregularly coralloid terminal elements, projections knob-like, digitate or clavate, 2–10(–20) × 2–5 (–9) µm, hyphae thin-walled, subhyaline to light brown in KOH. Pileus trama composed of hyaline, interwoven, partly gelatinized, thin- to moderately thick-walled (≤1 µm thick), 1.5–11 µm broad hyphae. Stipitipellis a cutis of parallel, cylindrical, ≤13 µm broad, thick-walled hyphae (≤6 µm thick), mostly brown in KOH, pigment parietal. Caulocystidia abundant in upper part of stipe, 18–50(60) × 7–12 µm, subcylindrical to narrowly clavate with an obtuse apex, sometimes branched, thick-walled (2–5 µm thick), subhyaline to light brown in KOH. Stipe trama composed of (sub)hyaline, parallel, thin- to thick-walled (≤4 µm thick), 2–8 µm broad hyphae. Clamp connections rare, seen only in stipe trama.

Chemical reactions — all parts of basidioma non-amyloid and non-dextrinoid except hyphae in cortical layer of stipe and caulocystidia, which are strongly dextrinoid.

Habitat — In forest, on dead twigs.

Distribution — Known only from the type locality in Singapore, Asia.

Remarks — *Gymnopus subrigidichorda* is characterized by a rufous brown striate-sulcate pileus with umbilicate center; incarnate to rufous brown lamellae attached to the pseudocollarium; sub-insititious minutely pubescent, brown stipe; abundant robust black rhizomorphs; basidiomata both growing on twigs and arising from rhizomorphs; non-hymeniform pileipellis (irregular cutis with lobed to coralloid terminal elements); ellipsoid, oblong, or lacrymoid spores; rare clamp connections (present only in stipe trama); clavate cheilocystidia with irregular apical projections; abundant thick-walled caulocystidia with obtuse apex; and dextrinoid hyphae in the stipe cortex. We consider it a good species. Our description pretty well agrees with the original description, except in spore size, which is significantly smaller (4.5–6.0 × 3.5–4.0 µm) in original description (Corner 1996).

Among other species in sect. *Androsacei* with clamp connections, black rhizomorphs, non-glabrous stipe, and colored (not white or cream) pileus and lamellae, *G. bactrosporus* has a smaller pileus (2.5–3.5 mm broad), hyaline-pilose stipe (under a hand-lens), longer (10–12 × 2.7–3.7 µm) and more elongated spores, well-developed pleurocystidia, and thin-walled caulocystidia. *Gymnopus brevistipitatus* has a smaller pileus (2–4 mm broad), shorter stipe (2–3 mm long), fewer lamellae (L = 7–9), and more elongated, narrower spores (2.5–3.7 µm wide). *Gymnopus campinaranae* has a smaller pileus (2–3 mm broad), thinner stipe (≤0.1 mm wide), and larger spores (9–13 × 3.2–4.5 µm). *Gymnopus fuscotramus* has free lamellae, shorter (≤135 mm long) and thinner rhizomorphs (≤0.5 mm wide), more elongated spores, gray-brown pileal and hymenophoral trama, and caulocystidia with thinner walls (0.5–2(–2.5) µm thick). *Gymnopus nigroimplicatus* has a pileus without central depression, narrower lamellae, shorter and thinner rhizomorphs, narrower spores (2.3–3.7 µm wide) and clamp connections present in all tissues (not only in stipe trama). *Gymnopus rhizomorphicola* has a shorter (±3 mm long), eccentric and curved stipe, fewer lamellae (L = 7–8), more elongated spores (Q = 2.1–2.9), and larger caulocystidia (45–190 × 6–26 µm). *Gymnopus rigidichorda* has creamy white mature lamellae and very narrow thinner (0.4–0.6 mm wide) rhizomorphs, differently shaped non-coralloid cheilocystidia, and narrower spores (3–3.5 µm wide). *Gymnopus thiersii* has rare or absent rhizomorphs, crowded lamellae, and lacks cheilocystidia. Because *G. subrigidichorda* has rare clamp connections (seen only in stipe trama) it could also be confused with a few species that lack clamp connections. *Gymnopus aporpohyphes* differs by a smaller (≤5 mm broad) and convex pileus with small umbo, whitish to pale avellaneous lamellae, narrower cheilocystidia (4–6.5 µm wide), and more elongated narrower (2.8–3.2 µm wide) spores. *Gymnopus pacificus* differs by a smaller pileus (5–6.5 mm broad), narrower stipe (0.2–0.3 mm wide), narrower spores (3.5–4.1 µm wide), and caulocystidia with thinner walls (0.5–1.5 µm wide). *Gymnopus tomentellus* differs by a smaller pileus (1–3 mm broad) without a central depression, smaller stipe (1–4 × 0.2–0.6 mm), larger and more elongated spores (11–11.5 × 4–5 µm), and caulocystidia with thinner walls (ca. 1 µm wide).

Additional new combinations in *Gymnopus*

Gymnopus aporpohyphes (Singer) Tkalčec & Mešić, **comb. nov.**

MycoBank MB 800668

≡ *Marasmius aporpohyphes* Singer, Sydowia 18: 333, 1965 ["1964"].

Gymnopus bactrosporus (Singer) Mešić & Tkalčec, **comb. nov.**

MycoBank MB 800669

≡ *Marasmius bactrosporus* Singer, Sydowia 18: 334, 1965 ["1964"].

Gymnopus brevistipitatus (Antonín) Tkalčec & Mešić, **comb. nov.**
MycoBank MB 800670
≡ *Setulipes brevistipitatus* Antonín, Mycotaxon 88: 71, 2003.

Gymnopus campinaranae (Singer) Mešić & Tkalčec, **comb. nov.**
MycoBank MB 800671
≡ *Marasmius campinaranae* Singer, Fieldiana, Bot. 21: 57, 1989.

Gymnopus pacificus (Singer) Tkalčec & Mešić, **comb. nov.**
MycoBank MB 800672
≡ *Marasmius pacificus* Singer, Beih. Nova Hedwigia 29: 95, 1969.

Gymnopus rhizomorphicola (Antonín) Mešić & Tkalčec, **comb. nov.**
MycoBank MB 800673
≡ *Setulipes rhizomorphicola* Antonín, Mycotaxon 88: 75, 2003.

Gymnopus rigidichorda (Petch) Tkalčec & Mešić, **comb. nov.**
MycoBank MB 800674
≡ *Marasmius rigidichorda* Petch, Trans. Brit. Mycol. Soc. 27(3-4): 138, 1945 ["1944"].

Gymnopus thiersii (Desjardin) Mešić & Tkalčec, **comb. nov.**
MycoBank MB 800675
≡ *Marasmius thiersii* Desjardin, Mycologia 79(1): 123, 1987.

Gymnopus tomentellus (Berk. & M.A. Curtis) Tkalčec & Mešić, **comb. nov.**
MycoBank MB 800676
≡ *Marasmius tomentellus* Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10(45): 298, 1868 ["1869"].

Acknowledgments

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An emendation of *Fusticeps* and two new species from the Brazilian Amazon Forest

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Abstract — Two new species, *Fusticeps lampadiformis* and *F. papillatus*, are described and illustrated. The description of *Fusticeps* is emended to accommodate smooth-spored species, and a key to the four *Fusticeps* species is provided.

Key words—freshwater fungi, anamorphic fungi, taxonomy

Introduction

Webster & Davey (1980) established *Fusticeps* for the single species *F. bullatus,* collected on submerged leaves in Malaysia. The most conspicuous feature of the surface ornamentation of the conidia is a projection that expands to a thin-walled domed cap; according to Webster & Davey (1980), the air entrapped between the projections may possibly aid conidia flotation. This character was used to include this genus in the aero-aquatic hyphomycetes (Fisher 1977, Goh & Hyde 1996, Hyde & Goh 1998). The genus remained monotypic until Matsushima (1993) described F. laevisporus collected on dead leaves in Ecuador. The original generic description — "Mycelium septate, dark. Conidiophores erect, septate, unbranched or occasionally branched. Conidia holoblastic, integrated, solitary, dark, transversely septate, epispore fractured into irregular scales which entrap air" — does not accommodate *F. laevisporus,* which has smooth conidia; the rhexolytic conidial secession is not mentioned and only the broad basal scar is described. However, several characters shared by *F. bullatus* and *F. laevisporus* (e.g., conidial ontogeny, secession, morphology) justify emending the generic description of *Fusticeps* to accommodate species with smooth conidia.

There are few records of this genus in the literature. It has been collected in Malaysia and Ecuador in lentic environments (Webster & Davey 1980;

Matsushima 1993) and in Poland in lentic and lotic environments (Orlowska et al. 2004, Orlowska et al. 2006, Czeczuga et al. 2007).

During study of anamorphic fungi associated with submerged litter in the Brazilian Amazon forest, *Fusticeps* was very well represented by collections of the two previously described species and two new species, described here as *F. lampadiformis* (with ornamented conidia) and *F. papillatus* (with smooth conidia).

Materials& methods

Collecting expeditions were made in two different sites in Amazon forest, near Belém County, Pará State: Parque Estadual de Utinga and Área de Proteção Ambiental Ilha do Combu. Submerged litter was collected from streams and placed in plastic bags containing humid paper towels. In the laboratory, the samples were removed from the plastic bags and incubated in Petri dishes with moistened paper towels at ambient temperature (about 24°C). Samples were examined within two weeks after collection, and then periodically, using a dissecting microscope. For microscopic identification the specimens were placed on glass slides containing PVL resin (polyvinyl alcohol, lactic acid, and phenol). Measurements and digital images were made using an Olympus microscope BX51 equipped with bright field and Nomarski interference optics and a microscopy image acquisition DP25 digital color camera. Specimens in glass slides and dry material were deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Fusticeps J. Webster & R.A. Davey, Trans. Br. Mycol. Soc. 75: 341, 1980; emend. J.S. Monteiro & Gusmão

Colonies on natural substratum effuse, olivaceous to olivaceous brown, dark to very dark brown. Mycelium mostly immersed in the substratum, composed of septate, brown to pale brown hyphae. Conidiophores macro- or micronematous, erect, septate, unbranched or occasionally branched, pale brown. Conidiogenous cells holoblastic, integrated, determinate, terminal, cylindrical, pale brown. Conidial secession rhexolytic. Conidia solitary, clavate to pyriform, transversally septate, smooth or with conspicuous ornamentation, pale brown. Teleomorph unknown.

Type species: *Fusticeps bullatus* J. Webster & R.A. Davey

Fusticeps lampadiformis J.S. Monteiro & Gusmão, **sp. nov.** Plate 1a–j

MycoBank MB 800031

Differs from *Fusticeps bullatus* in producing pyriform to lampadiform, 2–3-septate conidia.

Type: Brazil. Pará: Parque Estadual do Utinga, 1°25′S 48°27′W, on unidentified submerged leaves, 18 Jan 2012, J. S. Monteiro (**holotype**, HUEFS 42783).

Etymology: *lampas* (Latin), referring to the conidial shape.

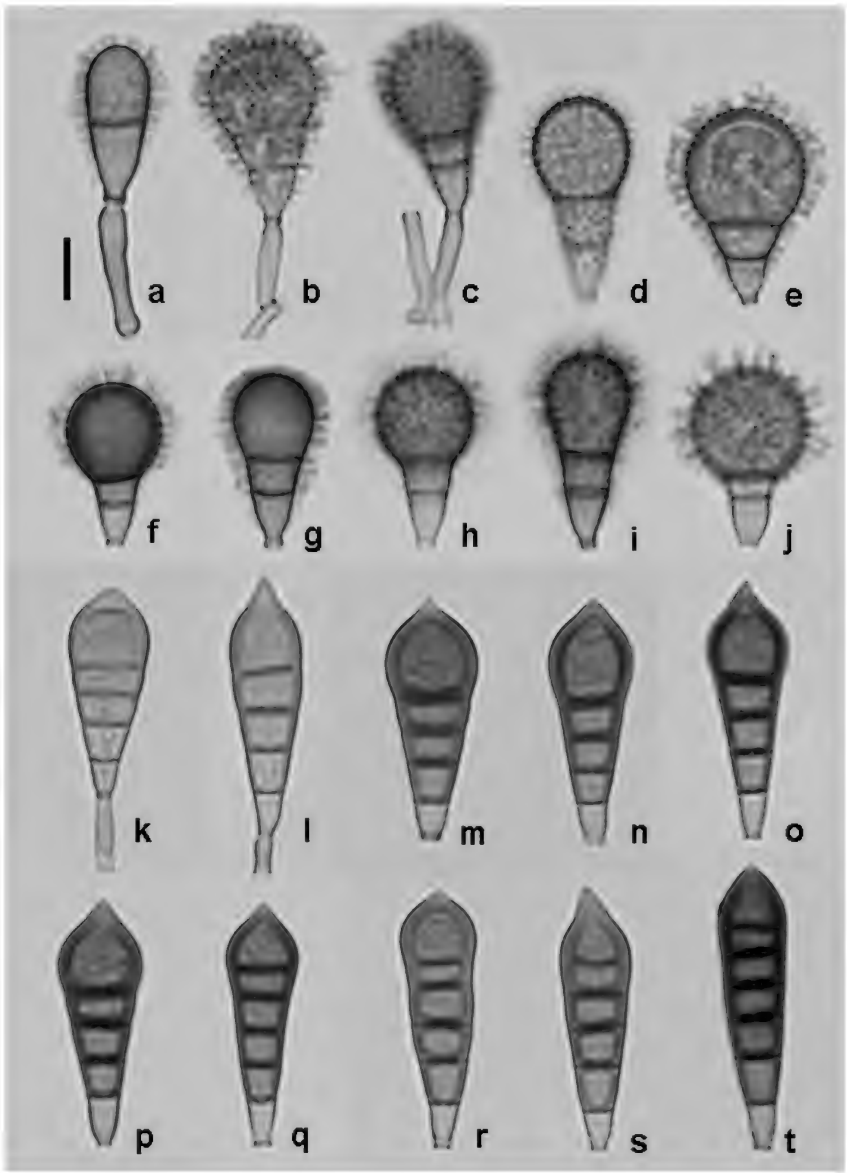

Plate 1.
Fusticeps lampadiformis: a. Immature conidia. b, c. Conidiogenous cell. d–j. Mature conidia.
Fusticeps papillatus: k, l. Conidiogenous cell. m–t. Mature conidia.
Scale bar = 10 µm.

Colonies on natural substratum effuse, olivaceous to olivaceous brown. Mycelium mostly immersed in the substratum, composed of septate, smooth, pale brown hyphae. Conidiophores micronematous, mononematous, unbranched or occasionally branched, straight, erect or slightly flexuous, septate, smooth, pale–brown, 22.5–62.5 × 2.5–5 µm. Conidiogenous cells monoblastic or rare polyblastic, integrate, determinate, terminal, cylindric, pale brown. Conidial secession rhexolytic. Conidia solitary, pyriform to lampadiform, 2–3 transversally septate, with cup-shape projections (3–5 × 1.5–2.5 µm) arising from the conidial wall, distal cell and intermediate cell brown, proximal cell pale brown, 21.5–35.5 × 12.5–18 µm; basal scar 2–3 µm.

Comments — *Fusticeps lampadiformis* is close to *F. bullatus*, which differs by conidia that are clavate, 3–4 septate, and have predominantly mushroom-shaped projections (Webster & Davey 1980).

Fusticeps papillatus J.S. Monteiro & Gusmão, **sp. nov.** Plate 1k–t

MycoBank MB 800032

Differs from *Fusticeps laevisporus* in producing clavate, 4–6 septate conidia with papillate apices.

Type: Brazil. Pará: Parque Estadual do Utinga, 1°25′S 48°27′W, on unidentified submerged leaves, 18 Jan 2012, J. S. Monteiro (**holotype**, HUEFS 42784).

Etymology: diminutive of *papula* (Latin), referring to the papilla at the conidial distal cell.

Colonies on the natural substratum effuse, dark brown. Mycelium mostly immersed in the substratum, composed of septate, smooth, brown to pale brown hyphae. Conidiophores micronematous, mononematous, unbranched or occasionally branched, grouped into 3–4, straight, erect or slightly flexuous, septate, smooth, pale–brown, 17.5–67.5 × 2.5–3.8 µm. Conidiogenous cells monoblastic, integrate, determinate, terminal, cylindric, brown to pale brown. Conidial secession rhexolytic. Conidia solitary, clavate, 4–6 transversally septate, smooth, distal cell thicker wall 1.5–2 µm, with a papilla (2–4 µm longer), distal cell and intermediate cells brown to pale brown, proximal cell pale brown, 35–45.5 × 11.5–15 µm; basal scar 2–3 µm.

Comments— *Fusticeps papillatus* is characterized by clavate, smooth, 4–6 septate conidia with a papilla at the apex of the distal cells. *Fusticeps laevisporus* is a comparable species but is distinguished by absence of a papilla and fewer conidial septa (Matsushima 1993).

Fusticeps bullatus J. Webster & R.A. Davey, Trans. Br. Mycol. Soc. 75: 342, 1980. Plate 2a–e

Colonies on the natural substratum effuse, dark. Mycelium mostly immersed in the substratum, composed of septate, smooth, pale brown hyphae.

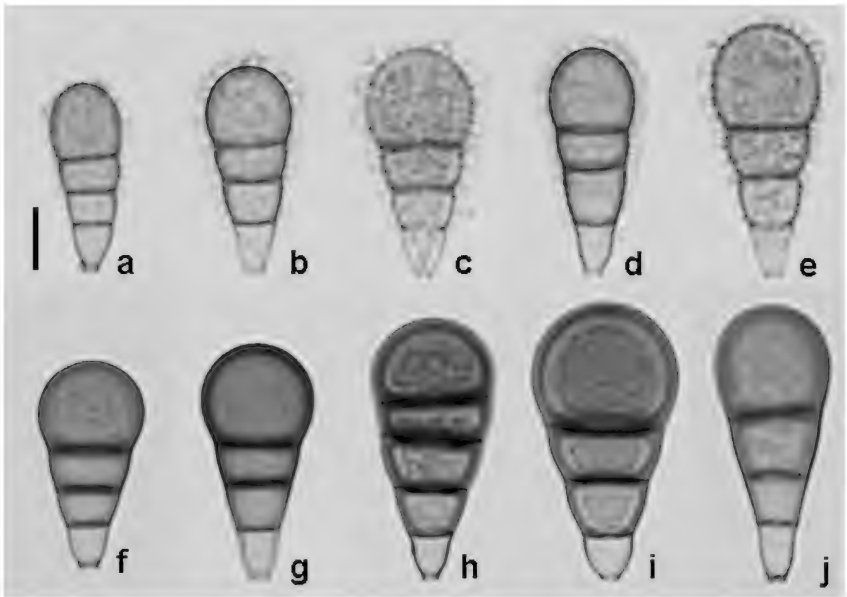

Plate 2. ***Fusticeps bullatus***: a–e. Mature conidia. ***Fusticeps laevisporus***: f–j. Mature conidia. Scale bar = 10 µm.

Conidiophores micronematous, mononematous, unbranched, septate, smooth, brown to pale brown 20–22.5 × 2.5–3.75 µm. Conidiogenous cells holoblastic, integrate, determinate, terminal, cylindric. Conidial secession rhexolytic. Conidia solitary, clavate, 2–3 transversally septate, with mushroom–shaped to cup–shaped projections (2.5 µm long) arising from the conidial wall, pale brown 23.5–34 × 10–13 µm; basal scar 2–3 µm.

Specimen examined: BRAZIL, Pará, Área de Proteção Ambiental Ilha do Combu, 1°29′S 48°25′W, on unidentified submerged leaves, 17 Jan 2012, J.S. Monteiro (HUEFS 42785).

Comments — *Fusticeps bullatus* is characterized by clavate, 3–4 septate conidia with mushroom-shaped to cup-shaped projections (Webster & Davey 1980). Our Brazilian specimens and Matsushima's (1993) Ecuadorian material, both from Amazon forest, have similar conidial dimensions and septation but differ slightly from the original Malaysian material (Table 1). Previously known from Malaysia, Ecuador, and Poland (Webster & Davey 1980, Matsushima 1993, Orlowska et al. 2004, Czeczuga et al. 2007); this is the first record for Brazil.

Fusticeps laevisporus Matsush., Matsush. Mycol. Mem. 7: 52, 1993. Plate 2f–j

Colonies on natural substratum effuse, dark. Mycelium mostly immersed in the substratum, composed of septate, smooth, pale brown hyphae.

Table 1. Synopsis of *Fusticeps* conidia

Species	Conidia			
	Size (µm)	Shape	Septa	References
F. bullatus	28–34 × 9–12	Clavate	3–4	Webster & Davey (1980)
	23.5–35 × 8–12.5	Clavate	2–3	Matsushima (1993)
	23.5–34 × 10–13	Clavate	2–3	This paper
F. laevisporus	30–44 × 9.5–13	Clavate	2–3	Matsushima (1993)
	25–40 × 10–20	Clavate	3–4	This paper
F. lampadiformis	21.5–35.5 × 12.5–18	Pyriform	2–3	This paper
F. papillatus	35.5–45.5 × 11.5–15	Clavate	4–6	This paper

Conidiophores micronematous, mononematous, unbranched, septate, smooth, brown to pale brown 20–47.5 × 2.5–3.75 µm. Conidiogenous cells monoblastic, integrate, determinate, terminal, cylindrical. Conidial secession rhexolytic. Conidia solitary, clavate, 3–4 transversally septate, smooth, distal cell with rounded apex and intermediate cells brown, proximal cell pale brown, 25–40 × 10–20 µm; basal scar 2–3 µm.

Specimen examined: BRAZIL, Pará, Parque Estadual do Utinga, 1°25′S 48°27′W, on unidentified submerged leaves, 18 Jan 2012, coll. J.S. Monteiro (HUEFS 42786).

Comments— *Fusticeps laevisporus* has smooth conidia and is comparable with *F. papillatus,* but differs by the rounded apex of the distal cell. Matsushima (1993) based his description of *F. laevisporus* on pure culture, and his conidia are narrower (9.5–13 µm) and have fewer septa (2–3) than our Brazilian material, but we consider the Ecuadorian and Brazilian collections to be conspecific. This species is a new record for Brazil; previously known from Ecuador and Poland (Matsushima 1993, Orlowska et al. 2006).

Key to *Fusticeps* species

Conidial measurements and morphologies are summarized in Table 1.

1. Conidia smooth. 2
1a. Conidia with conspicuous ornamentation 3
2. Conidia 2–4-septate, apex rounded . . . . . *F. laevisporus*
2a. Conidia 4–6-septate, apex papillate . . . . . *F. papillatus*
3. Conidia pyriform, 2–3-septate . . . . . *F. lampadiformis*
3a. Conidia clavate, 3–4-septate . . . . . *F. bullatus*

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The authors thank Dr. Marc Stadler (Department Microbial Drugs, Helmholtz Centre for Infection Research – Germany) and Dr. R.F. Castañeda Ruiz (INIFAT – Cuba) for their great contribution in improving the manuscript. The authors extend thank to Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support and the "Programa de Pós-graduação em Biologia de Fungos –

PPGBF/UFPE". This study was supported by The National Council for Scientific and Technological Development (CNPq) (Grant for LFP Gusmão, Proc. 303924/2008–0).

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Two *Ropalospora* lichens new to mainland China

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Abstract —The genus *Ropalospora* is reported from mainland China for the first time; *R. chlorantha* is new to China, while *R. phaeoplaca* is new to mainland China.

Key Words —taxonomy, corticolous, fungi, *Ropalosporaceae*

Introduction

Ropalospora A. Massal., first described in 1860, belongs to the *Ropalosporaceae* (Kirk et al. 2008). The genus is characterized by black prothallus, dark brown to black lecideine apothecia, *Fuscidea*-type ascus, bacilliform conidia, and multiseptate ascospores (Ekman 1993, Brodo et al. 2001, Kantvilas 2004). *Ropalospora* includes six species worldwide: *R. chlorantha*, *R. hibernica* (P. James & Poelt) Tønsberg, *R. lugubris* (Sommerf.) Poelt, *R. phaeoplaca*, *R. rossii* Øvstedal, and *R. viridis* (Tønsberg) Tønsberg (Ekman 1993, 1996; Tønsberg 1993; Brodo et al. 2001; Øvstedal et al. 2001; Smith et al. 2009; Fryday & Coppins 2012).

In China, *Ropalospora phaeoplaca* has been reported only from Taiwan (Aptroot & Sparrius 2003). Here we present records of *R. phaeoplaca* and *R. chlorantha* from mainland China.

Materials & methods

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University) and KUN-L (Lichen Section, Cryptogamic Herbarium of Kunming Institute of Botany, Academia Sinica). Morphological and anatomical characters were examined under a stereomicroscope (COIC XTL7045B2) and polarizing microscope (Olympus CX41). The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C and microcrystal test (MCT) with GE solution (Orange et al. 2010). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Ropalospora chlorantha (Tuck.) S. Ekman, Bryologist 96: 586 (1993) FIGS 1–2

MORPHOLOGY — Thallus corticolous, crustose, pale brown, discrete or continuously verrucose areolate, thin, esorediate; prothallus black; medulla with obvious crystals that are soluble in K and C. Apothecia lecideine, sessile, 0.4–0.8 mm diam.; disc black, concave; margin black, often flexuous. Exciple externally brown, internally hyaline to straw-colored, without crystals; epihymenium brown to black; hymenium hyaline; hypothecium hyaline, with oil droplets. Asci clavate, 30–50-spored, *Fuscidea*-type; ascospores hyaline, needle-shaped, 3–5-septate, with one attenuate end, curved, 26–35 × 1–2 μm;

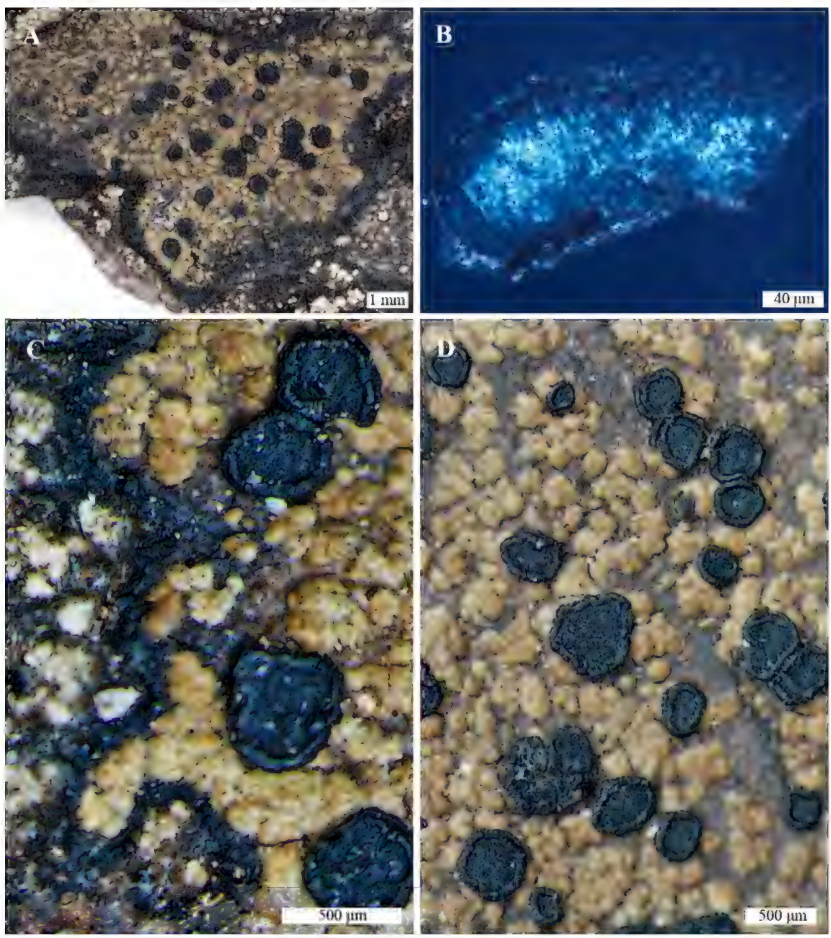

FIG. 1 ***Ropalospora chlorantha*** (20100315a, SDNU). A: Thallus; B: Crystals in thallus; C: Prothallus; D: Apothecia.

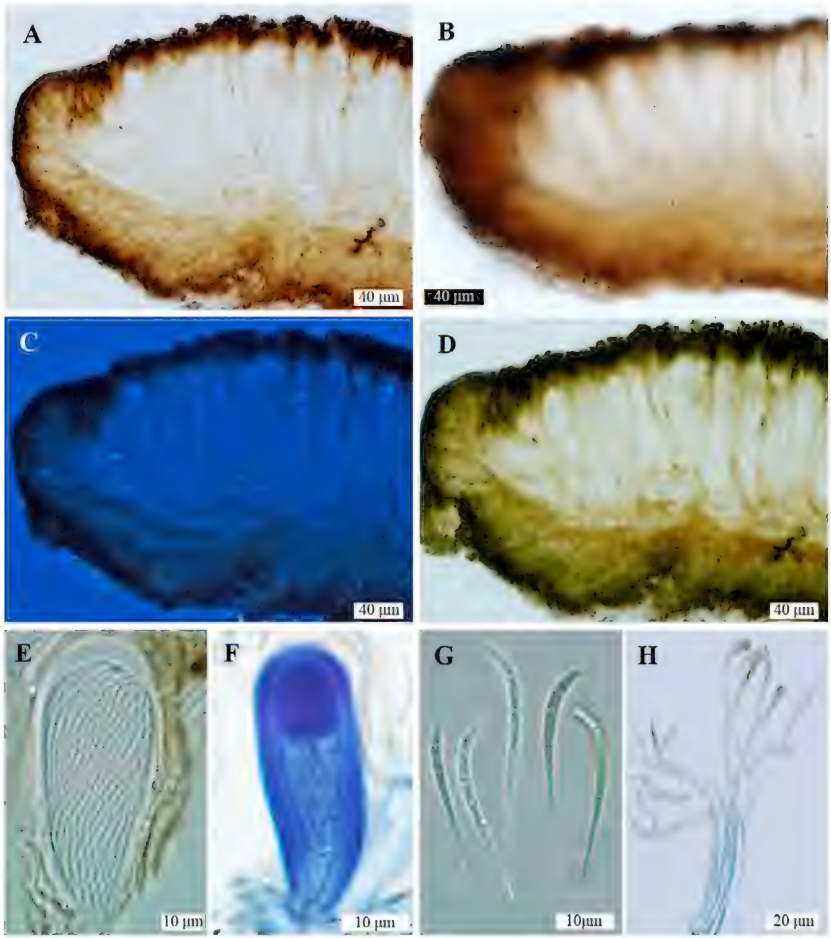

FIG. 2 ***Ropalospora chlorantha*** (20100315a, SDNU). A: Apothecium section; B: Apothecium section, showing oil droplets in hypothecium; C: Apothecium section, showing exciple without crystals; D: Apothecium section, showing K reaction (turning green) of epihymenium and outer exciple; E: Ascus and ascospores; F: Amyloid reaction of ascus; G: Ascospores; H: Paraphyses.

paraphysis 1.5–2.0 µm diam., branched in upper part and slightly thickened at top. Pycnidia not observed.

CHEMISTRY — Thallus and medulla K–, C–, KC–, P–. Epihymenium and exciple K+ green, C–. No lichen substances detected by TLC or MCT.

DISTRIBUTION — *Ropalospora chlorantha* has been reported from Canada, USA, and Bhutan (Ekman 1993, Aptroot & Feijen 2002). New to China.

SPECIMEN EXAMINED: **CHINA. Yunnan**, Lijiang, Mt. Laojun, alt. 4000 m, on bark, 5 Nov 2009, Q. Tian 20100315a (SDNU).

Comments —*Ropalospora chlorantha* is characterized by the corticolous and esorediate thalli, the exciple without crystals, the >30-spored asci, and perlatolic acid. However, we detected no lichen substance in this specimen from China.

Ropalospora phaeoplaca (Zahlbr.) S. Ekman, Op. Bot. 127: 128 (1996) Figs 3–4

Morphology — Thallus corticolous, crustose, grayish-white, smooth to verrucose areolate, without soredia; prothallus not seen; medulla with obvious

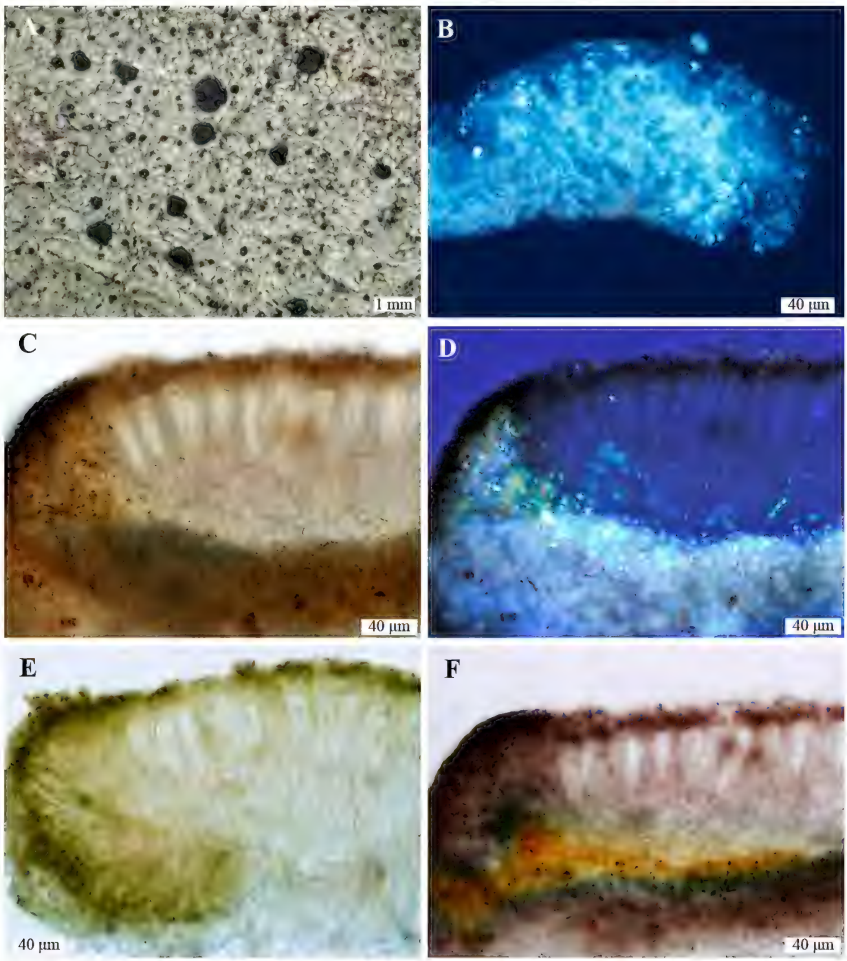

Fig. 3 ***Ropalospora phaeoplaca*** (20105161, SDNU). A: Thallus; B: Crystals in thallus; C: Apothecium section, showing oil droplets in apothecium; D: Apothecium section, showing exciple with crystals; E: Apothecium section, showing K reaction (turning green) of epihymenium and outer exciple; F: Apothecium section, showing C reaction (turning green, then purple) of epihymenium and outer exciple.

FIG. 4 ***Ropalospora phaeoplaca*** (20105161, SDNU). A: Amyloid reaction of asci; B: Ascus and ascospores; C: Paraphyses; D: Ascospores; E: MCT result (GE solution); F: TLC result (R = Reference).

crystals that are soluble in K and C. Apothecia lecideine, sessile, 0.4–0.9 mm diam.; disk black, slightly convex; margin black, smooth or flexuose. Exciple brown externally, inside pale brown to hyaline, with abundant crystals that are soluble in K and C; epihymenium brown; hymenium hyaline; hypothecium hyaline, with oil droplets. Asci clavate, 10–16-spored, *Fuscidea*-type; paraphysis simple, slightly branched at top; ascospores hyaline, needle-shaped, 5–6 (–8)-septate, with one attenuate end, curved, 30–47 × 2–3 µm. Pycnidia not observed.

Chemistry —Thallus and medulla K–, C–, KC–, P–. Epihymenium and outer exciple K+ green, C+ green then turn to purple. An unknown lichen substance was detected by TLC and MCT.

Distribution — *Ropalospora phaeoplaca* has been reported from Japan and Taiwan (Ekman 1996, Aptroot & Sparrius 2003). New to mainland China.

Specimens examined: **CHINA. Fujian**, Longyan, Mt. Huanglianyu, alt. 1400 m, on bark, 29 Oct, 2010, H.Y. Wang 20105161, X.R. Kou 20105920 (SDNU). **Guizhou**, Kaili, Mt. Leigong, alt. 1600 m, on bark, 23 Aug 2010, D.F. Jiang 20103297, Z.L. Huang 20103179, 20103155, 20103128, L.L. Zhang 20103148a, 20103148b (SDNU); Tongren, Mt. Fanjing, alt. 2000 m, on bark, 2 Nov 2009, Z.J. Ren 20128007 (SDNU). **Yunnan**, Chuxiong, Mt. Zixi, alt. 2100 m, on bark, 28 Aug 1994, L.S. Wang 94–15646 (HKAS).

Comments — *Ropalospora phaeoplaca* is closely related to *R. chlorantha*, but differs in having minute K-soluble crystals in the exciple and usually eight (occasionally ≤16) spores per ascus (Ekman 1996). The Chinese specimens have abundant K-soluble crystals in the exciple, 10–16-spored asci, and an unknown lichen substance.

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The authors thank Dr. A. Aptroot (ABL Herbarium, Soest, the Netherlands) and Dr. Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for presubmission reviews. This study was supported by Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province, and the National Natural Science Foundation of China (31170187 & 31270059).

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South Florida microfungi: a new species of *Ellisembia* (hyphomycetes) with new records from the U.S.A.

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Abstract — *Ellisembia mercadoi* sp. nov., collected on rachides of dead leaves of *Sabal palmetto* in southwestern Florida, U.S.A., is described and illustrated. The fungus is characterized by cylindrical, determinate conidiogenous cells and verruculose, subfusiform to narrowly obclavate or obclavate-rostrate, 7–16 distoseptate conidia often with remnants of an apical mucilaginous sheath. Similarities and differences with morphologically related species within the genus are discussed. *Berkleasmium leonense*, *Ceratosporella basibicellularia*, *Hermatomyces tucumanensis*, *Monodictys capensis*, and *Selenosporella perramosa* are newly recorded from the U.S.A.

Key words — anamorphic fungi, palm fungi, *Sporidesmium*, taxonomy

Introduction

The southern tip of the Florida peninsula, with its humid subtropical climate, extensive habitat diversity, and distinctive vegetation that includes a high percentage of tropical plant species, is likely to harbor a rich but still under-explored mycobiota. In the course of our continuing studies on saprobic microfungi occurring in the area, particularly those hyphomycetous anamorphs associated with dead plant debris (Delgado 2008a, b, 2009, 2010), a distinct and unusual species of *Ellisembia* Subram. was found. The fungus agrees well with the generic concept originally introduced by Subramanian (1992) for *Sporidesmium*-like taxa with distoseptate conidia and conidiophores with or without proliferating conidiogenous cells. However, it differs morphologically from previously described *Ellisembia* species and therefore is proposed here as new. Five other hyphomycetes are also recorded for the first time from the U.S.A.

Materials & methods

Samples of dead leaves belonging to two palm tree species commonly found in south Florida, the cabbage palm (*Sabal palmetto* (Walter) Lodd. ex Schult. & Schult.

f.,) and the Everglades or paurotis palm (*Acoelorrhaphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc.) were collected in forested areas of Broward and Collier counties during 2007–2010. They were cut in smaller pieces, air-dried, and placed in plastic bags for further processing and examination following Cannon & Sutton (2004). Slides were prepped using lacto-cotton blue as mounting medium. Fungal structures were examined, measured at 1000×, and photographed using an Olympus BX-45 microscope. All specimens examined, including the type specimen of *Ellisembia mercadoi* as well as semi-permanent slides, are deposited in the U.S. National Fungus Collections Herbarium (BPI).

Taxonomy

Ellisembia mercadoi G. Delgado, **sp. nov.** FIGS 1–9

MycoBank MB# 804032

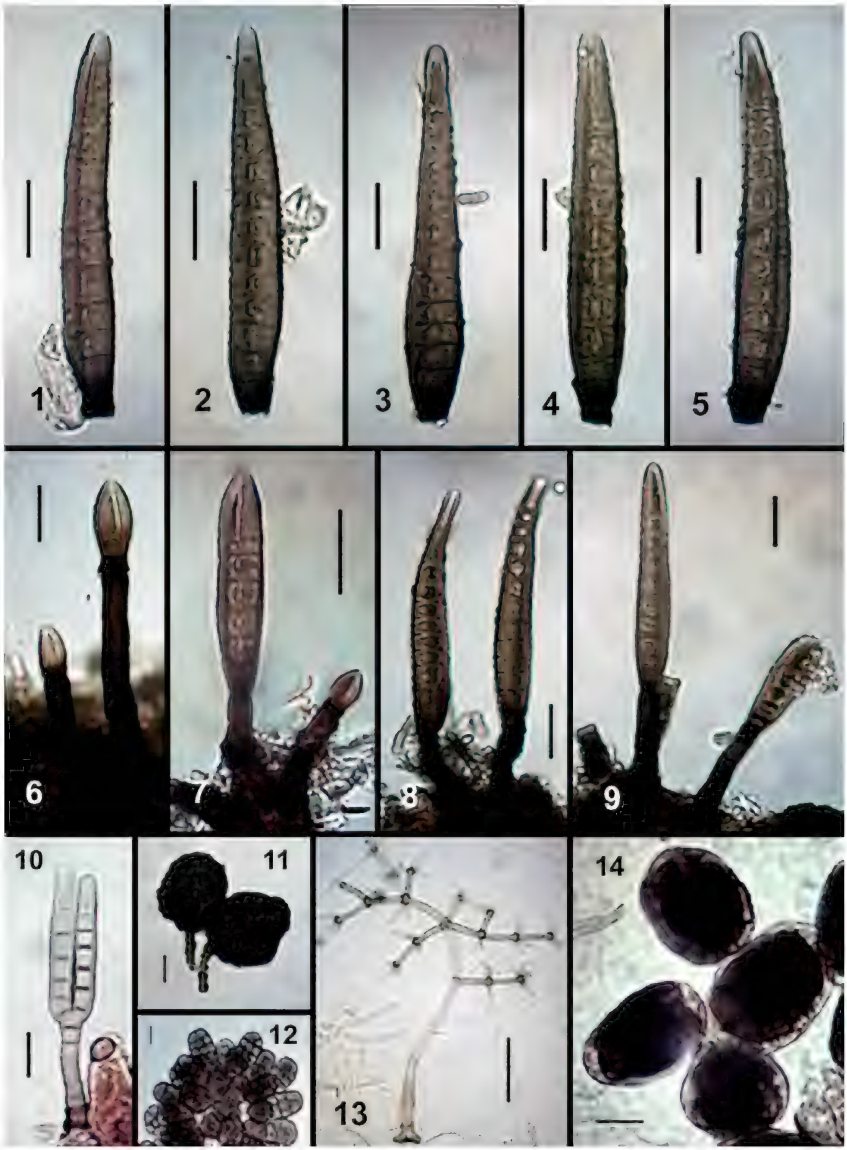
Differs from *Ellisembia fusiformis* in having cylindrical conidiogenous cells and longer verruculose, brown, obclavate-rostrate, conidia with an apical mucilaginous sheath, and from *E. crassispora* in having smaller conidia, determinate conidiogenous cells, and shorter conidiophores.

TYPE — U.S.A. Florida, Collier Co., Naples, 26°3′35″N 81°41′43″W, on rachis of dead leaf of *Sabal palmetto*, 23.XI.2007, coll. G. Delgado (**Holotype:** BPI 880515F).

ETYMOLOGY — Latin, *mercadoi*, in honor of the Cuban mycologist Dr. Angel Mercado-Sierra (1937–2008) for his many contributions to the study of tropical and worldwide hyphomycetes.

COLONIES on natural substrate effuse, hairy, inconspicuous. MYCELIUM predominantly immersed in the substrate, composed of branched, septate, smooth-walled, pale brown to brown hyphae, 1–2.5 µm wide. STROMATA none or rudimentary, dark brown to blackish brown. CONIDIOPHORES macronematous, mononematous, single or aggregated in small groups, simple, erect, straight or flexuous, cylindrical, 2–5-septate, smooth, dark brown to dark reddish brown, 33–76 × 6–9 µm, 10–14 µm wide at the swollen base. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, cylindrical, determinate, dark brown to dark reddish brown; apex truncate, often darkened. CONIDIAL SECESSION schizolytic. CONIDIA holoblastic, solitary, dry, acrogenous, straight or slightly curved, subfusiform to narrowly obclavate, older conidia often obclavate-rostrate, 7–16-distoseptate, sometimes abruptly constricted at one distoseptum, brown, paler toward the apex, verruculose, 63–168 × 12–15 µm; apex usually rounded or slightly acute and often with remnants of an apical mucilaginous sheath; basal cell conico-truncate, dark brown to black. TELEOMORPH unknown.

ADDITIONAL SPECIMEN EXAMINED — **U.S.A. FLORIDA**, COLLIER CO., Naples, 26°3′35″N 81°41′43″W, on rachides of dead leaves of *Sabal palmetto*, 23.XI.2007, coll. G. Delgado (BPI 880521L).



Figs 1–14. ***Ellisembia mercadoi*** (holotype, BPI 880515F): 1–5. Conidia. 6. Conidium initials. 7. Young conidia. 8–9. Conidiophores and conidia. ***Ceratosporella basibicellularia*** (BPI 884153A): 10. Conidiophore and conidium. ***Monodictys capensis*** (BPI 880518D): 11. Conidiophores and conidia. ***Berkleasmium leonense*** (BPI 884154G): 12. Sporodochium with conidia. ***Selenosporella perramosa*** (BPI 884156D): 13. Conidiophore, conidiogenous cells and conidia. ***Hermatomyces tucumanensis*** (BPI 884154D): 14. Conidia. Scale bars: 1–8 = 20 μm; 9 = 15 μm; 10–12; 13 = 30 μm; 14 = 10 μm.

Discussion

The genus *Ellisembia* has recently undergone numerous additions (Ma et al. 2008, 2010, 2011, McKenzie 2010, Rajeshkumar et al. 2012, Ren et al. 2012, Santa Izabel et al. 2013) totaling 59 entries in the Mycobank Database (www.mycobank.org). Wu & Zhuang (2005), however, had previously enlarged the original concept of Subramanian (1992) after placing *Imicles* Shoemaker & Hambl. (= *Imimyces* A. Hern. Gut. & B. Sutton) (Shoemaker & Hambleton 2001) into synonymy with *Ellisembia*. Consequently, a small group of additional *Sporidesmium*-like taxa producing lageniform, doliiform, or ovoid percurrent proliferations and distoseptate conidia could be transferred to *Ellisembia* following this broad concept, but only three combinations were formally made: *E. bambusae* (M.B. Ellis) W.P. Wu, *E. carrii* (Morgan-Jones) W.P. Wu, and *E. leptospora* (Sacc. & Roum.) W.P. Wu. This left three species in *Imicles* (*I. aquatica* (Cabello et al.) Shoemaker & Hambl., *I. heterocateniformis* (Matsush.) Shoemaker & Hambl., *I. pseudobambusae* (P.M. Kirk) Shoemaker & Hambl.) and one in *Imimyces* (*I. hollowayensis* A. Hern. Gut. & B. Sutton (Hernandez-Gutierrez & Sutton 1997) as possible members of *Ellisembia*. Seifert et al. (2011), however, retained *Imicles* as a separate genus, and in the case of *I. heterocateniformis* (≡ *Sporidesmium heterocateniforme* Matsush.) (Hernandez-Gutierrez & Sutton 1997, Matsushima 1987), a reexamination of the type specimen is certainly necessary to clarify its correct taxonomic placement.

Ellisembia mercadoi is characterized by dark brown to dark reddish brown, cylindrical conidiophores often arising from a rudimentary stroma and producing determinate, not proliferating, conidiogenous cells. The conidium initial is distinctly mitriform, pale brown, and delimited by an incipient black basal cell with the outer wall apparently showing signs of breakage resembling longitudinal splits from the pointed apex downwards. After elongation, the young conidia become cylindrical or subcylindrical with a slightly acute apex, and when fully developed they are usually subfusiform or narrowly obclavate but also obclavate-rostrate with a well-defined conico-truncate basal cell. Remnants of an apical mucilaginous sheath were often visible near the conidial apex as traces of mucilage or as short, lateral and subapical, thread-like filaments. A well defined cap as seen in many other *Ellisembia* species (McKenzie 1995, Wu & Zhuang 2005) was not observed, although further collections may provide evidence of its presence.

Among the accepted *Ellisembia* species, *E. mercadoi* is morphologically similar to *E. fusiformis* (Nees & T. Nees) Subram. (Ellis 1958) in conidial shape and width, number of septa, the presence of a black conico-truncate basal cell, and determinate conidiogenous cells. However, *E. fusiformis* has dark reddish brown smooth-walled non-rostrate shorter conidia (≤136 µm long) without an apical mucilaginous sheath, and the conidiogenous cells are not darkened and

taper abruptly toward the apex. *Ellisembia crassispora* (M.B. Ellis) Subram. (Ellis 1958, Wu & Zhuang 2005) also has verruculose, obclavate and rostrate conidia with a conico-truncate basal cell but clearly differs in longer conidiophores (≤300 µm long) with up to 3 percurrent proliferations and dark brown to blackish brown larger conidia (100–250 × 18–30 µm) with up to 22 distosepta and also lacking a mucilaginous sheath.

Additional new records from U.S.A.

Berkleasmium leonense M.B. Ellis, More Dematiaceous Hyphomycetes: 69, 1976. Fig. 12

Specimen examined: **U.S.A.. Florida**, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°6′25″N 80°13′19″W, on rachides of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (BPI 884154G).

Ceratosporella basibicellularia Matsush., Matsush. Mycol. Mem. 7: 45, 1993.Fig. 10

Specimen examined: **U.S.A.. Florida**, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°6′25″N 80°13′19″W, on petiole of dead leaf of *Acoelorrhaphe wrightii* and overgrowing the surface of old hysterothecia, 30.V.2010, coll. G. Delgado (BPI 884153A).

Hermatomyces tucumanensis Speg., Anal. Mus. nac. Hist. nat. B. Aires 13: 446, 1911. Fig. 14

Specimen examined: **U.S.A.. Florida**, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°6′25″N 80°13′19″W, on rachides of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (BPI 884154D).

Monodictys capensis R.C. Sinclair, Boshoff & Eicker, Mycotaxon 59: 359, 1996. Fig. 11

Specimens examined: **U.S.A.. Florida**, Collier Co., Naples, 26°3′35″N 81°41′43″W, on rachides of dead leaves of *Sabal palmetto*, 23.XI.2007, coll. G. Delgado (BPI 880518D, 880521E, 880515C); Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°6′25″N 80°13′19″W, on rachides and petioles of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (BPI 884154F, 884155A).

Selenosporella perramosa (W.B. Kendr. & R.F. Castañeda) R.F. Castañeda, Mycotaxon 109: 69, 2009. Fig. 13

Specimens examined: **U.S.A.. Florida**, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°6′25″N 80°13′19″W, on rachides, petiole, petiole-lamina junction and segments of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (BPI 884151C, 884152D, 884154B, 884156D).

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Aureoboletus zangii (*Boletaceae*), a new species from China

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Abstract —*Aureoboletus zangii* sp. nov. is described from central China based on morphological and molecular analysis. This species is found fruiting in association with hardwood trees. It is similar to the European *A. gentilis* and Asian *A. thibetanus* but is characterized by the yellowish brown or reddish golden basidiomata, glutinous pileus with subtomentum, and viscid stipe. LSU sequence analysis supports the new species in *Aureoboletus* (*Boletaceae*). Photographs, line drawings, and a phylogenetic tree showing relationships with closely allied taxa are provided.

Key words — Chinese fungal diversity, *Boletales*, taxonomy

Introduction

Two *Aureoboletus* species have previously been reported from China, *A. thibetanus* (Pat.) Hongo & Nagas. (Ying & Zang 1994) and *A. reticuloceps* M. Zang et al. (Zang et al. 1993), although the latter species has been transferred to *Boletus* (Wang & Yao 2005). Worldwide only 10 *Aureoboletus* species are recognized (Kirk et al. 2008). Although Pouzar named *Aureoboletus* with the type *A. gentilis* (Quél.) Pouzar in 1957 (Corner 1972, Kirk et al. 2008), the genus was not accepted for some time (Smith & Thiers 1971, Corner 1972, Singer 1986, Šutara 2005). After recent molecular analyses by Binder (1999) and Binder & Hibbett (2006) helped resolve the taxonomy of the genus, Klofac (2010) presented a world monograph in which he transferred 13 additional taxa to *Aureoboletus* and presented a species key (including some critical species in other genera). The morphological characters differentiating this genus from other bolete genera include: medium-small sized basidiomata, a pileus that is both viscid and subtomentose, yellow-colored tubes and pores, a hymenophoral surface more or less depressed around the stipe apex, a cylindrical to slightly swollen glutinous stipe that is never reticulate nor glandulose, and (most species) ectomycorrhizal with frondose trees (Klofac 2010).

Materials & methods

Macroscopic and microscopic descriptions were based on fresh and dried material respectively. Color codes follow Kornerup & Wanscher (1961). For microscopical observations, slides were revived in 5% KOH solution and Melzer's reagent. The observation of the pileipellis structure was performed following Wang & Verbeken (2006). Micro-morphological features were examined under a Nikon E400 microscope at 1000× magnification and drawings were made through a drawing tube (Y-IDT) installed on the microscope. For further explanation of basidiospore data, see Yang (2000). All specimens examined were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

Total DNA was extracted from silica-dried materials using a modified CTAB procedure by Doyle & Doyle (1987). The polymerase chain reaction (PCR) was performed using the primer pairs LROR/ LR5 to amply the ribosome larger subunit (nrDNA-LSU) region of the nuclear ribosomal DNA (White et al. 1990). Sequencing of both strands was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services CO., Ltd, Shanghai). The sequences have been submitted to GenBank (accession numbers JQ734420–734422).

Taxonomy

Aureoboletus zangii X.F. Shi & P.G. Liu, **sp. nov.** FIGS 1, 2

MycoBank 564683

Differs from *Aureoboletus gentilis* and *A. thibetanus* by its concolorous stipe and pileus and its shorter basidiospores.

Type: China, Shaanxi Province, Baoji City, Meixian County, Haoping Town, Dali Village, 34°05′N 107°42′E, alt. 1200 m, 4.IX.2010, X.F. Shi 654 (**Holotype**, HKAS63217; GenBank JQ734420).

Etymology: *zangii* is in honor of Prof. Mu Zang (28 Dec. 1930–10 Nov. 2011), a distinguished Chinese mycologist who founded the Cryptogamic Herbarium at Kunming Institute of Botany, Academia Sinica (KUN-HKAS).

Macrocharacters — Basidiomata small to medium, scarcely fleshy. Pileus at first hemispherical then expand to convex, 3–4 cm in diam; surface glutinous when fresh, grayish orange to yellowish brown or reddish golden to light brown (6B3, 5D5–5E5, 6C4–6D5); with concolorous subtomentum, without sterile margin. Stipe 5.5–7 × 0.5–0.8 cm, cylindrical or clavate, usually tapering towards the base, mostly concolorous with the pileus or more reddish, light brown to brownish orange (7C6, 6D5), sometimes darkening towards the base, violet-brown (11E8), sometimes changing to reddish-white (9A2) or violet-brown (10F7) when bruised, distinctly viscid especially in wet weather, entirely fistulose and showing white mycelium at the base. Context 0.2–0.3 cm thick, pale yellow or orange-white (2A3, 6A2), always more or less pinkish under the cuticle, unchanging when bruised or exposed to air. Tubes 0.3–0.5 cm deep, vivid yellow at first and then olive yellow, grayish yellow or vivid yellow (2C6,

Fig 1. *Aureoboletus zangii* (Holotype).

2B7, 2A8, 2A6), unchanging when exposed to air. Pores 1–2 per mm, bright yellow or concolorous with tubes, unchanging, depressed around the stipe. Odor not distinctive. Taste mild.

Microcharacters — Basidiospores [80/3/3] (10–)10.5–11.0 × (4.0–)4.5–5.0 ⌈m, Q = (2.1–)2.2–2.4(–2.5), Q_m = 2.23 ± 0.09, elongate elliptical, yellowish-olivaceous in 5% KOH, thin-walled, sometimes with tiny oil drops; yellow to yellowish-brown in Melzer's. Basidia 30–38 × 11–14 µm, clavate, with 4 sterigmata. Pleurocystidia 35–65 × 11–18 µm, narrowly clavate, yellow in 5% KOH and lacking any incrustations. Cheilocystidia 35–42.5 × 11–17 µm, similar to pleurocystidia. Caulocystidia absent. Hymenophoral trama divergent, with hyphae 4–5 µm in diameter, thin-walled. Pileipellis a layer consisting of mostly inflated hyphae tending to repent under the gelatinous layer, slightly brownish in 5% KOH, 7–10 µm in diameter.

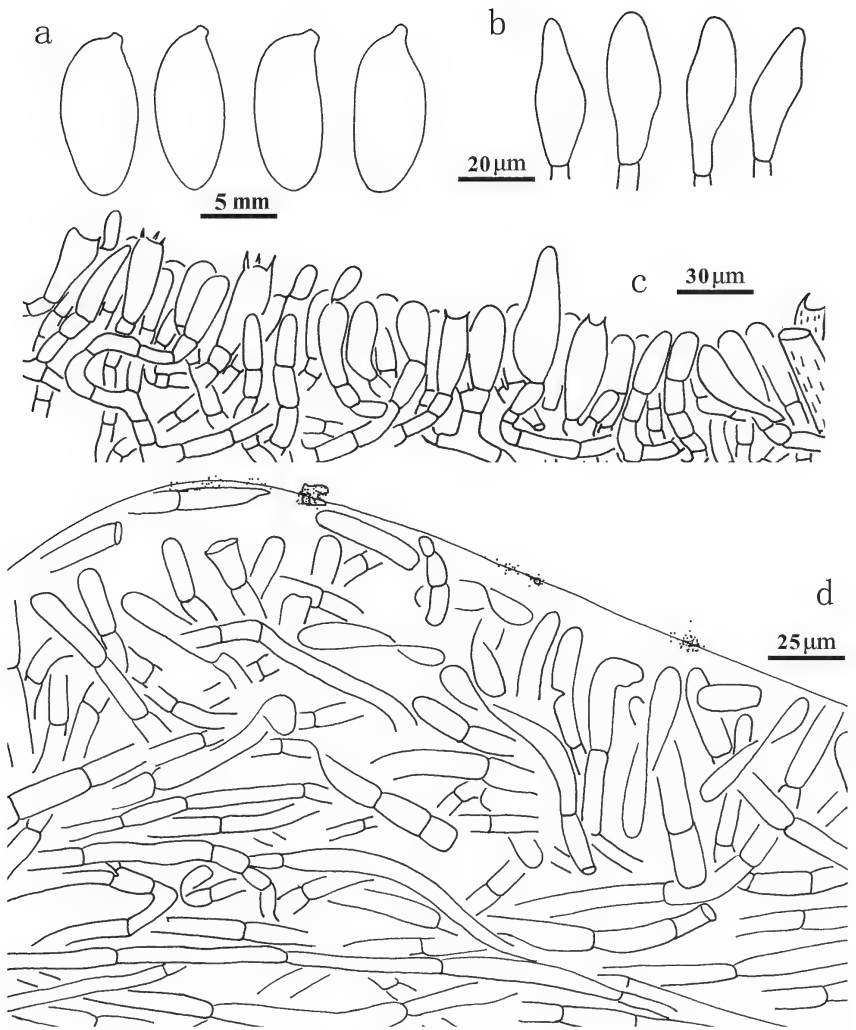

Fig 2. *Aureoboletus zangii* (Holotype). a. Basidiospores; b. Cystidia; c. Hymenium; d. Pileipellis.

Ecology & Distribution —Solitary, rarely gregarious, widespread in central China, on the ground, in subtropical broad-leaved mixed forests dominated by *Cyclobalanopsis* and *Quercus*, alt. 1200–1400 m.

Additional specimens examined — **China. Guizhou**, Daozhen: near Yangxi Town, 28°50′N 107°36′E, alt. 1200 m, 28.VII.2010, X.F. Shi 394 (HKAS 63220; GenBank JQ734422); Yangxi Town, Dashahe Conservation Area, 28°50′N 107°36′E, alt. 1400 m, 27.VII.2010, X.F. Shi 388 (HKAS 63219; GenBank JQ734421).

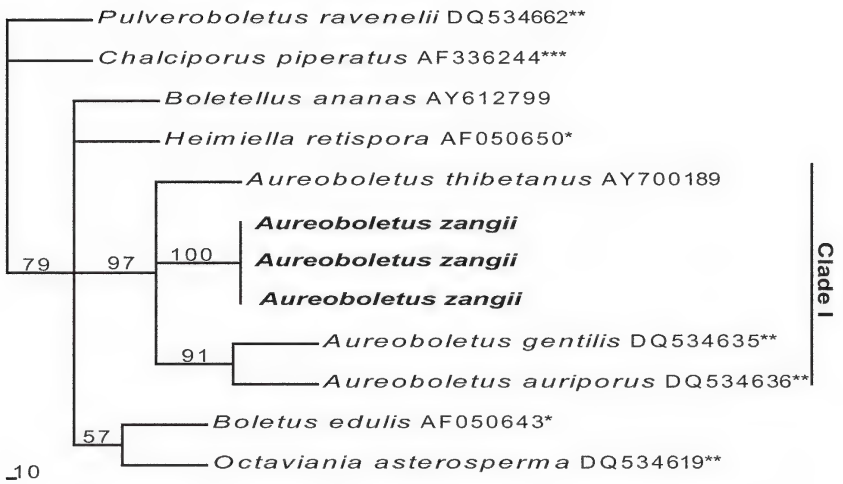

FIG 3. The MP tree from a phylogenetic analysis of LSU sequences of *Boletaceae*. Numbers above branches are bootstrap values (>50%). Sequences obtained in this study are presented in bold front; * = sequences by Binder & Fischer (1997); ** = sequences by Binder (2006); *** = sequences by Wang et al.(2002); all other sequences were downloaded from Genbank.

Phylogenetic results

Three samples were used for phylogenetic analysis. *Aureoboletus zangii* fell into the well-supported Clade I in FIG. 3. Phylogenetic analysis of nLSU supports *A. zangii* should be a member of the genus *Aureoboletus* along with the European species *A. gentilis* and the Asian species *A. thibetanus*.

Discussion

The phylogeny confirms the new taxon as sister to *A. gentilis* and *A. thibetanus*. *Aureoboletus zangii* is similar to the European *A. gentilis* in its viscid reddish orange cap and viscid stipe, but the stipe of the new species is concolorous with its pileus, while in *A. gentilis* the stipe is bright yellow with just pinkish to brownish tinges in places. *Aureoboletus zangii* might also be confused with the Asian *A. thibetanus,* which can be distinguished by its reddish brown shrunken cap and yellowish white stipe. Anatomically, the *A. gentilis* spores are with largest (≤15 µm long) with those of *A. thibetanus* around 12µm long and those of *A. zangii* around 10 µm long. In addition, the *A. zangii* cystidia are <65 µm long while those of *A. gentilis* and *A. thibetanus* are ≤70 µm long (Klofac 2010; Yang et al. 2003). Moreover, the outer surface of *A. thibetanus* is characteristically covered by a 5–8 µm thick layer of a strongly refractive yellow substance that is quickly and completely dissolved in 5% KOH (Yang et al. 2003).

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Molecular characterization of *Colletotrichum* species causing soybean anthracnose in Argentina

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Abstract —Twenty-six isolates obtained from soybean crops (*Glycine max*) with typical anthracnose symptoms were identified as *Colletotrichum truncatum* (73 %) and *C. destructivum* (26 %). Their genetic relationships were studied using the AFLP method. A UPGMA phenogram divided the strains into two clusters corresponding with the two species. Genetic distances based on association coefficient were 0.71–0.89 among the 18 *C. truncatum* strains and 0.67–1 among the eight *C. destructivum* strains. Genetic variability within species, measured in terms of percentage of polymorphic loci, was high (<90%). Only two isolates showed 100% similarity, suggesting high intraspecific variability.

Key words —*Glomerella glycines*, teleomorph, genetic diversity

Introduction

Soybean (*Glycine max* (L.) Merr.) is the most important crop in Argentina, with late-season diseases caused by *Septoria glycines, Cercospora kikuchii, Phomopsis sojae, Colletotrichum destructivum,* and *C. truncatum* causing major losses (Carmona et al. 2009, 2011).

The two anthracnose pathogens most frequently associated with soybean are *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore and *C. destructivum* O'Gara, [= *Glomerella glycines* Lehman & F.A. Wolf]. The traditional characterization and identification of *Colletotrichum* species has relied on morphological differences (Sutton 1992). Molecular markers constitute the current choice to obtain diagnostic characters as an aid for species-level identification and to evaluate genetic diversity. The amplified fragment length polymorphism (AFLP) method (Vos et al. 1995) is frequently used to monitor the identity of an isolate or to determine the degree of similarity among isolates, owing to certain advantages over other techniques (i.e. high level of identified polymorphism, high reproducibility, relative technical simplicity; Briad et

al. 2000). The taxonomy and nomenclature of the anthracnose pathogens (*Colletotrichum* spp.) is confusing (Hyde et al. 2009 a) and have recently been summarised by Hyde et al. (2009b), incorporating data obtained by DNA analysis and phylogenetic systematics.

Physiological studies have been performed on *C. truncatum* isolates obtained from soybean plants in Argentina (Levin et al. 2007, Ramos et al. 2010), but the genetic structure of anthracnose fungi associated with the Argentinean soybean crop is not available. Our study aimed to evaluate genetic variability and genetic relationships within *Colletotrichum* species obtained from symptomatic soybean plants in Argentina.

Materials & methods

Fungal Isolation

Diseased soybean plants showing typical anthracnose symptoms were collected from different geographic locations in Argentina. The isolates were obtained from lesions of stems and pods, using the procedure of Levin et al. (2007).

All fungal strains used in this survey were deposited at the Herbarium and Culture Collection of the Departamento de Biodiversidad, Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires (BAFC). Table 1 shows the collection locality and BAFC accession number of each strain used.

Table 1. *Colletotrichum truncatum* and *C. destructivum* fungal strains studied

Species	Collection locality*	Strains tested #
C. truncatum	Godeken, SF, Arg.	C1 (3093)
	Cerro Azul, SF, Arg.	C2 (3094)
	Armstrong, SF, Arg.	C4 (3096), C5 (3097)
	Cruz Alta, CO, Arg.	C6 (3098)
	Fac. Agronomía CABA, Arg.	C7 (3099)
	Lobos, BA, Arg.	C8 (3100), C9 (3101)
	Saenz Peña, CH, Arg.	C10 (3102)
	Salto, BA, Arg.	C14 (3396)
	Las Lajitas, SA, Arg.	C15 (3397)
	Piamonte, SF, Arg.	C26 (3398)
	Otamendi, BA, Arg.	C24 (3399), C32 (3448)
	Las Varillas, SF, Arg.	C25 (3400)
	Fac. Agronomía CABA, Arg.	C29 (3443), C30 (3444)
	Venado Tuerto, SF, Arg.	C31 (3447)
C. destructivum	Urdinarrain, ER, Arg.	C16 (3434)
	Chivilcoy, BA, Arg.	C17 (3435)
	Río Cuarto, CO, Arg.	C18 (3436)
	9 de Julio, BA, Arg.	C19 (3437)
	Barranca Yaco, SF, Arg.	C21 (3439)
	Piamonte, SF, Arg.	C23 (3441)
	Chivilcoy, BA. Arg.	C27 (3401), C28 (3442)

* Abbreviations: BA = Buenos Aires Province; CABA = Ciudad Autónoma de Buenos Aires; CH = Chaco Province; CO = Córdoba Province; ER = Entre Ríos Province; LR = La Rioja Province; SA = Salta Province; SF = Santa Fé Province; Arg.: = Argentina.

Numbers in parentheses correspond to the BAFC culture number (see Materials & methods).

Table 2. AFLP primers used in this study

Primers	Sequence 5´-3´
E+AC	GAC TGC GTA CCA ATT CAC
E+AA	GAC TGC GTA CCA ATT CAA
E+ACG	GAC TGC GTA CCA ATT CAC G
E+AAC	GAC TGC GTA CCA ATT CAA C
E+AAG	GAC TGC GTA CCA ATT CAA G
M+A	GAT GAG TCC TGA GTA AA
M+G	GAT GAG TCC TGA GTA AG
M+C	GAT GAG TCC TGA GTA AC
M+T	GAT GAG TCC TGA GTA AT

* E = *EcoRI*; M = *MseI*.

DNA extraction

Actively growing mycelia were obtained and then ground in liquid nitrogen (Ramos et al. 2000). Genomic DNA was extracted following Gottlieb & Lichtwardt (2001). Quality control and quantification of genomic DNA was carried out by agarose gel (0.8% w/v) electrophoresis and by comparison with a DNA molecular-size standard (Lambda EcoRI/HindIII, Promega Corp.). Gels, containing ethidium bromide, were photographed under UV light.

AFLP

The AFLP methodology was carried out on 250 ng of genomic DNA using the AFLP® Analysis System for Microorganisms Primer Kit (Invitrogen) as described in the instructions manual with minor modifications (Gottlieb et al. 2005). Selective primers used herein, containing one, two or three additional bases, were combined as in Table 2. All PCR amplifications were performed in a Techne Progene thermal cycler.

Polyacrylamide gel electrophoresis conditions were conducted as in Gottlieb et al. (2005). A 30-330-bp AFLP® DNA Ladder (Invitrogen) size marker was included twice in each electrophoresis run. Thus, the size of scored AFLP bands ranged from 90 to 330 bp. AFLP bands were visualized using the Silver Sequence ™ DNA Sequencing System (Promega). Air-dried gels were digitalized and visually analyzed using the Adobe Photoshop TM (Adobe Systems, Mountain View, CA, USA).

Data analysis

Each AFLP band, regardless of its relative intensity, was considered as a dominant allele at only one locus. The data, as either present (1) or absent (0), were extracted as a table. Monomorphic bands (bands present in all individuals of a species) were discriminated within each species and across the entire data set. The binary matrix was analyzed with the program NTSYS-PC version 2.02i (Rohlf 1993). The unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis was performed based on the simple matching (SM), Jaccard (J), and Dice (D) association coefficients (Sneath & Sokal 1973). Phenograms showing similarity relations were generated by the same program, with the tree display option (TREE). Correlation cophenetic coefficients (r) were calculated for each association coefficient (Sokal & Rohlf 1962). A cophenetic correlation (r) of 0.8 was considered a good fit. A three-dimensional graphic was obtained with the Principal Coordinates ordination method (Gower 1966).

The percentage of polymorphic loci (P = number of polymorphic loci/ number of loci analyzed) for each primer combination was calculated.

Results

Twenty-six isolates obtained from soybean crops that showed typical anthracnose symptoms were identified as *Colletotrichum truncatum* (73%) and *C. destructivum* (26%), based on their morphological characteristics (Sutton 1992).

Characterization of AFLP markers

Six primers (Table 3) that produced high numbers of clear, reproducible bands by AFLP amplification were selected. The analysis of the six primers yielded a total of 812 bands that appeared consistently in all experiments among the amplified fragments of the 26 isolates. The size of the amplified bands ranged from 50 to 550 bp but we analyzed only bands from 90 to 330 bp.

The total number of AFLP bands (polymorphic and monomorphic) obtained and the percentage of polymorphic loci for each selective primer combination in two fungal species assayed are described in Table 3. All primers detected a high number of polymorphic bands within species and a high percentage of polymorphic loci was obtained.

Table 3. Total AFLP bands (polymorphic and monomorphic) detected for each primer combination in each assayed fungal species with polymorphic loci percentages (P%).

Primer combination	Species	Total N° of bands	Polymorphic bands	Monomorphic bands	P%
M+T/E+ACG	*C. truncatum*	73	65	8	89
	C. destructivum	81	74	7	91
M+C/E+AAG	*C. truncatum*	117	111	6	95
	C. destructivum	111	109	2	98
M+A/E+AAG	*C. truncatum*	95	93	2	98
	C. destructivum	56	51	5	91
M+A/E+ACG	*C. truncatum*	73	71	2	97
	C. destructivum	100	96	4	96
M+G/E+ACG	*C. truncatum*	82	80	2	97
	C. destructivum	54	52	2	96
M+A/E+AAC	*C. truncatum*	103	82	21	79
	C. destructivum	56	48	8	86

Only 41 (7.6%) of the bands obtained in *C. truncatum* and 28 (6.1%) in *C. destructivum* were monomorphic. Genetic variability within species, measured in terms of percentage of polymorphic loci, was high: *C. truncatum* (P = 92%) and *C. destructivum* (P = 94%).

The UPGMA phenogram (Figure 1) grouped all strains into two defined clusters with a SM coefficient of 0.6 between them. The main cluster included

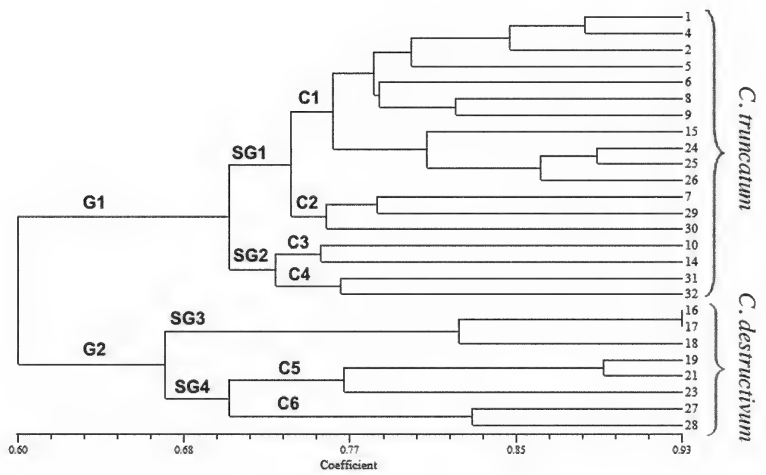

FIGURE 1. UPGMA phenogram showing relationships of *Colletotrichum truncatum* and *C. destructivum* isolates based on the simple matching (SM) association coefficient estimated from AFLP loci.

the 18 isolates identified as *C. truncatum* (FIGURE 1, G1), whereas the other (FIGURE 1, G2) included the eight isolates identified as *C. destructivum*. There was no direct correlation between molecular genotype and geographic origin.

Genetic distance and cluster analysis

The phenogram obtained using the UPGMA method was constructed based on Simple Matching (SM) coefficients. Little distortion occurred while constructing this phenogram, as implied by the value of the correlation cophenetic coefficient (r = 0.91903).

The main group (FIGURE 1, G1) comprised two subgroups (SG1 and SG2), one with 14 isolates and two conjuncts (C1 and C2), and the other (SG2) with two conjuncts (C3 and C4), each with two isolates. Group 2, formed by *C. destructivum* isolates, showed two subgroups, SG3 formed by three isolates (16, 17 and 18), and SG4, which included conjunct C5 and C6 formed by the rest of isolates belonging to *C. destructivum*. The relatedness of the 18 isolates of *C. truncatum* was 0.71–0.89, whereas that among the 8 isolates of *C. truncatum* was 0.67–1. Only two isolates (16 and 17, SG3) showed 100% similarity.

The three-dimensional graphic generated by the ordination technique distinguished two groups (FIGURE 2, GI and GII) and showed nearly the same relations between isolates as the phenogram (FIGURE 1). The first group included the 18 isolates belonging to *C. truncatum*, which are slightly differentiated in

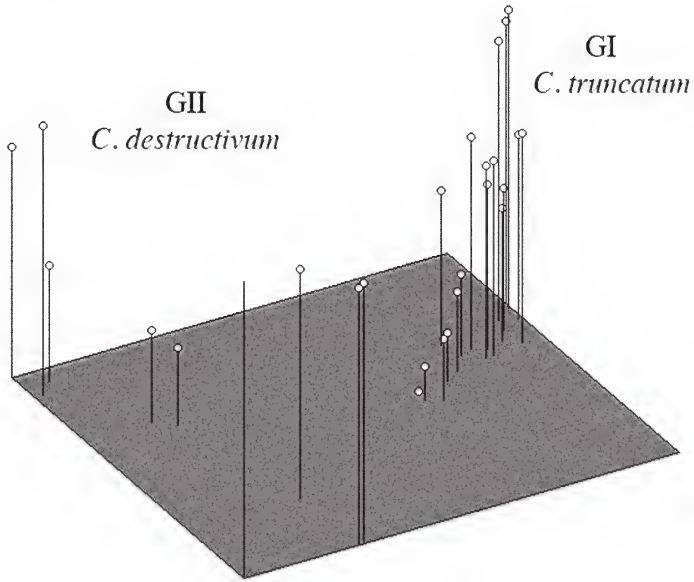

FIGURE 2. Three-dimensional graphic obtained with the Principal Coordinates ordination technique.

axis 1 and largely separated in the other two axes. The second group showed the seven isolates of *C. destructivum* largely separated in axis 1 and moderately differentiated in axes 2 and 3. Group GI included isolates of SGI and SGII of the phenogram and group GII comprised the isolates of SGIII and SGIV.

Discussion

C. truncatum and *C. destructivum* are highly variable pathogens in Argentina. Sharma (2009) also reported a high degree of variability among different isolates of *C. truncatum* studied through Random Amplified Polymorphic DNA (RAPD) analysis.

Perithecia of the *C. destructivum* teleomorph (the state referred to in dual nomenclature as *Glomerella glycines*) are frequently found in the fields of Argentina, indicating that the sexual mechanism is an important source of variability in the populations of this species. However, sexual recombination in most *Colletotrichum* species is rare in nature (Wharton & Diéguez-Uribeondo 2004; Hyde et al. 2009b).

Nevertheless, in our study, we found a high degree of variability in the populations analyzed. Alternatives to sexual recombination for genetic interaction among fungi are heterokaryosis and mitotic recombination (Esser

& Kuenen 1967). In many *Colletotrichum* populations, the only means of exchanging genetic material between two strains is mainly or exclusively vegetative, by means of anastomosis and heterokaryosis (Correll et al. 2000). These processes have been studied in some *Colletotrichum* species (Vaillancourt 1994; Correll et al. 1993; Elmer 2001; Nitzan et al. 2002; Rodriguez-Guerra et al. 2003; Barcelos et al. 2011). Sant'Anna et al. (2010) related genetic diversity analyzed by RAPD with the presence of vegetative compatibility groups in *C. truncatum*, and concluded that the parasexual process might be an important factor to increase the genetic variability in the pathogen.

Almost the entire soybean crop area of Argentina is cultivated under no-tillage systems. The crop debris remaining on the soil surface provides optimum conditions for the active development of reproductive structures of *C. truncatum* and *C. destructivum*. Any event of mutation or sexual or vegetative recombination is enhanced by the high reproductive rate occurring in these conditions. Gene flow and migration forces seem to be relatively unimportant due to the weak dispersal ability of the spores, as both conidia and ascospores are immersed in a mucous matrix, which prevents dispersal, by wind. The only possibility of migration to long distances is by means of seed transmission. Nevertheless, both species are not frequent seed pathogens in the Pampean Region.

The level of genetic variability within populations of plant pathogens provides an indication of their adaptability of pathogen in overcoming plant resistance and other measures used to control the pathogen, such as the application of fungicides in agro-ecosystems (McDonald et al. 1989; McDonald & Linde 2002). In our study, the high genetic diversity indicates increasing fitness of the population.

This paper constitutes the first report of genetic variability in *C. truncatum* and *C. destructivum* isolates collected from different soybean growing areas in Argentina.

Acknowledgments

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A new species of *Cantharomyces* (*Laboulbeniales*, *Ascomycota*) from the Netherlands

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Abstract — This paper describes and illustrates *Cantharomyces elongatus* sp. nov., a parasitic fungus from *Syntomium aeneum* (*Staphylinidae*, *Oxytelinae*, *Euphaniini*). *Syntomium* is a new host genus for *Cantharomyces*. Comments on its position among related taxa are given.

Key words — insect-associated fungi, morphology, taxonomy

Introduction

Laboulbeniales are obligate ectoparasitic ascomycetes that live associated with arthropods, mostly insects. At present the order comprises about 2050 species in 140 genera (Rossi & Santamaría 2012). Systematic and taxonomic contributions are available for several countries in western Europe and North America.

The genus *Cantharomyces* Thaxt. includes 28 species (Tavares 1985, Huldén 1983, Majewski 1990, Rossi & Santamaría 2000), characterized by a receptacle consisting of three superposed cells, a compound antheridium subtending a simple or variably branched primary appendage, and a perithecium having four to five cells in each vertical row of outer wall cells (Thaxter 1931, Majewski 1994). Detailed information on *Cantharomyces* and its morphology and position among other *Laboulbeniales* is given in Thaxter (1890, 1896, 1908, 1931), Tavares (1985), Majewski (1990, 1994), and Santamaría (2003).

The host range of *Laboulbeniales* on *Staphylinidae* and other *Coleoptera* has been studied by Frank (1982), who listed 17 *Cantharomyces* species. In addition to *Staphylinidae*, *Cantharomyces* species have been reported from *Dryopidae*, *Limnichidae*, and *Hydrophilidae* (Tavares 1985). Based on recent taxonomic insights in *Staphylinidae* from Newton & Thayer (2005) and Bouchard et al. (2011), four tribes of the *Staphylinidae* are host to *Cantharomyces*: *Blediini*

and *Oxyteliini* (subfam. *Oxytelinae*), and *Aleocharini* and *Oxypodini* (subfam. *Aleocharinae*) (Thaxter 1890, 1896, 1908, 1931, Sugiyama 1973, Frank 1982, Huldén 1983, Tavares 1985, Majewski 1990, 1994, Rossi & Santamaría 2000, Santamaría 2003).

This paper presents a new species of *Cantharomyces*, collected from a staphylinid beetle belonging to a fifth and so far unreported host tribe.

Materials & methods

The host was found by sifting soil from an ash forest on clay. Infected material was dried, pinned, and identified by O. Vorst (using Lohse 1964). Screening for infection and removal of thalli was done at 50× magnification. Thalli were transferred with insect pin 0 and embedded in Amann solution (Benjamin 1971). Cover slips were ringed with transparent nail varnish. Both insect specimen and the examined microscope slides are deposited at the National Herbarium of Belgium (BR). Drawings and measurements were made using an Olympus BX51 light microscope with drawing tube, digital camera and AnalySIS Five imaging software (Soft Imaging System GmbH). Differences in the proportions of basal cell and suprabasal cell were analyzed using the ratios R_L = length basal cell / length suprabasal cell and R_W = width basal cell / width suprabasal cell. Average ratios are given for adult thalli, including minimum and maximum values in parenthesis.

Host taxonomy follows Newton & Thayer (2005) and Bouchard et al. (2011).

Taxonomy

Cantharomyces elongatus Haelew. & De Kesel, **sp. nov.** PLATE 1

MycoBank MB 800871

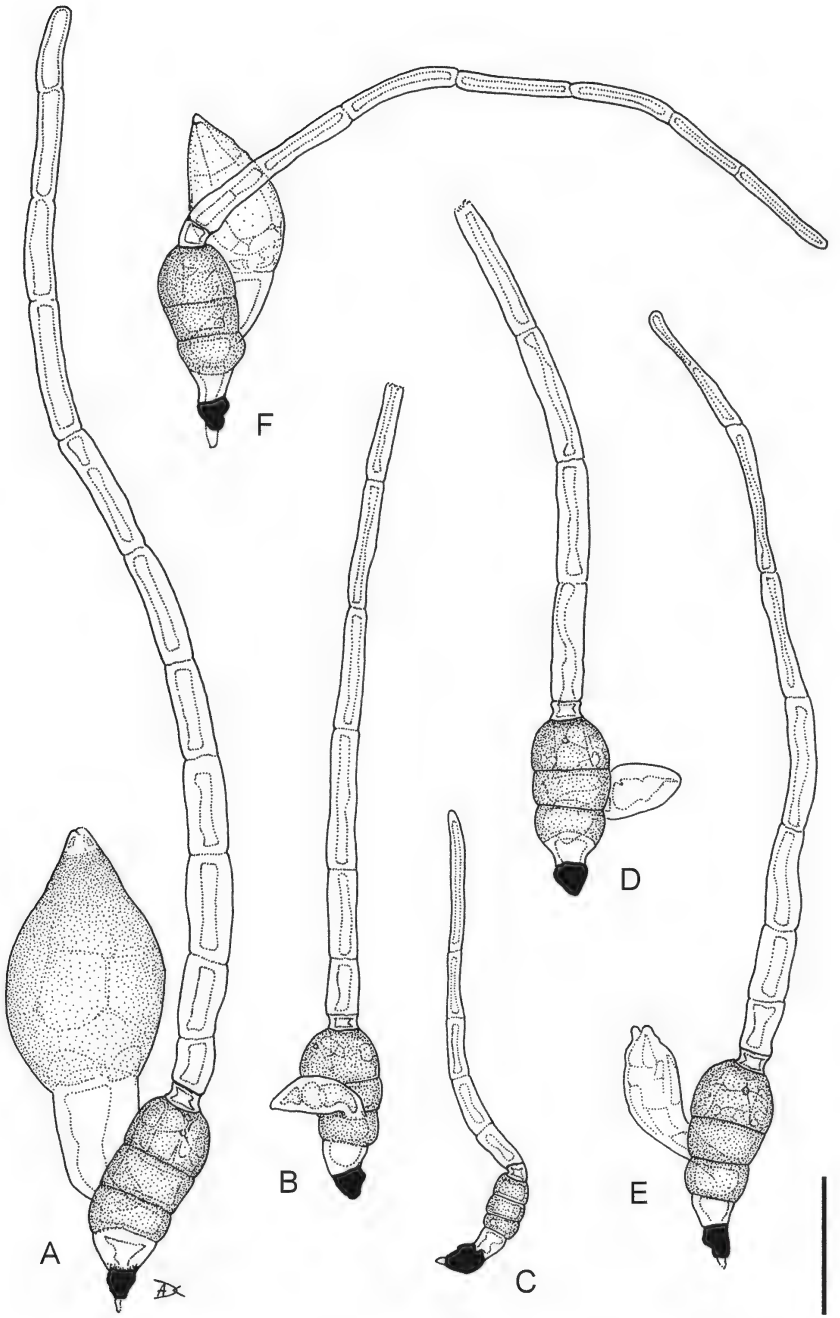
Differs from *Cantharomyces orientalis* by its single long, strictly unbranched, straight primary appendage with the basal cell > 2.5× longer and wider than the suprabasal cell.

TYPE: The Netherlands, Prov. Gelderland, Rha, 52°02.9′N 6°08.5′E, on *Syntomium aeneum* (Müller, 1821) (*Coleoptera*, *Staphylinidae*, *Oxytelinae*, *Euphaniini*), 13 Oct 2001, leg. O. Vorst, slides Haelewaters 69a (**holotype**, 2 mature and 4 immature thalli, found on left elytron and abdominal tergites, BR MYCO 173753-26), Haelewaters 69b (**isotype**, 1 mature and 2 immature thalli found on elytra, BR MYCO 173752-25), and Haelewaters 69c (**isotype**, 2 mature and 2 immature thalli found on elytra, BR MYCO 173751-24).

ETYMOLOGY: from the Latin adjective *elongatus* = elongate, referring to the long, unbranched primary appendage.

THALLUS 145–175 µm long from foot to perithecial apex. Receptacle 50–60 µm long, cell I obtriangular, 22–30 µm long, cell II and cell III with similar shape, slightly pigmented, not blackened, broader than long, cell III somewhat longer than cell II. PRIMARY APPENDAGE composed of a basal cell, a small

PLATE 1. *Cantharomyces elongatus* from *Syntomium aeneum*. A. Mature thallus from elytrum (holotype Haelewaters 69a). B–D. Immature thalli with perithecial primordium (holotype slide Haelewaters 69a). E. Immature thallus (isotype slide Haelewaters 69b). F. Submature thallus (isotype slide Haelewaters 69c). Scale bar = 50 µm.


F
A
B
C
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E

suprabasal cell and a series of elongate cells. Basal cell of primary appendage in the same axis as cell II and III, apically convex, slightly more pigmented than or concolorous with cells II and III, 23 × 30 µm (length × width), basal septum with cell III straight. Suprabasal cell relatively flattened and small [R_L = 2.88 (2,77–3,17), R_W = 2.81 (2,58–2,96)], 6.5–10 µm long, trapezoidal, slightly constricted, supporting a straight, 300–415 µm long unbranched series of 7–11 hyaline, thick-walled, elongate cells of 25–40 µm each. Antheridium located around a central core in the basal cell of primary appendage, with subapical opening on the latter's posterior side. Perithecium 72–96 × 40–57 µm, ovoid, symmetric, widest in the middle, tapering upwards, moderately pigmented; apex pointed, hyaline, poorly differentiated. Cell VI born laterally on cell II, 41–47 × 22–30 µm, hyaline. Ascospores not seen.

Additional specimens examined: **THE NETHERLANDS, Prov. Utrecht,** Utrecht (Amelisweerd), 52°04′N 5°09′E, on *Syntomium aeneum*, 11 Nov 1990, leg. O. Vorst, slides Haelewaters 145a (1 damaged thallus from right elytron, BR MYCO 173750-23) and Haelewaters 145b (3 immature thalli found on prosternum, BR MYCO 173749-22).

Discussion

Cantharomyces elongatus is easily recognized by its very long and unbranched primary appendage with a relatively large basal cell and small trapezoidal suprabasal cell. Most of the time, even in a dried state, this long structure is found undamaged, indicating its robustness. In many genera of *Laboulbeniales* damaged appendages are known to regenerate in an erratic or at least atypical way. Among all examined specimens of *C. elongatus* we found several thalli with a normally regenerated, i.e. unbranched, appendage. Atypical regeneration of the appendage may be infrequent in this taxon since we found only one specimen with a bifurcate regenerated appendage (on its fourth appendage cell). Whenever a branched or aberrant appendage is seen in this taxon, one should look carefully for traces of damage to the appendage.

Cantharomyces elongatus was found on the pronotum, elytra, and abdominal tergites and does not seem to be morphologically variable on these different regions.

Cantharomyces elongatus is related to *C. orientalis* Speg., a very variable species having a primary appendage that is ramified above its suprabasal cell. Occasionally, however, *C. orientalis* bears a more or less short, unbranched appendage. In such cases, *C. orientalis* can still be easily distinguished from *C. elongatus* by its proportionally longer and wider suprabasal cell. In *C. elongatus* the basal cell is at least 2.5× longer and 2.5× wider than the suprabasal cell. Based on data from Belgian material and illustrations in Santamaría 2003 and Majewski 1994, this proportion is significantly smaller for *C. orientalis*, R_L = 1.33 (0.85–1.96) and R_W = 1.24 (0.95–2.16). The combination of the appendage

characteristics of length, branching, and relative height of the suprabasal cell should be enough to separate *C. orientalis* from *C. elongatus*.

Moreover, although *C. elongatus* and *C. orientalis* parasitize staphylinids from the same subfamily (*Oxytelinae*), they infect hosts belonging to different tribes: *C. elongatus* infects hosts of tribe *Euphaniini* and *C. orientalis* hosts of tribe *Oxytelini*.

Cantharomyces robustus T. Majewski shows many differences from *C. elongatus*: the blackening on the dorsal side of cell II, the ramified, shorter appendage, the inflated and rounded basal cell of the appendage (with antheridium), as well as large perithecial basal cells.

The new species is easily separated from the *Cantharomyces* species with an unbranched primary appendage: *C. bordei* F. Picard, *C. denigratus* Thaxt., and *C. italicus* Speg., based on the extreme length and construction of its primary appendage. *Cantharomyces bordei* has an unbranched, but short primary appendage. Furthermore, it is reported from *Limnichus* spp. (*Coleoptera*, *Limnichidae*) and exhibits a very short and constricted cell VI as well as a relatively massive perithecium (124–130 µm long, Santamaría 2003). *Cantharomyces denigratus* and *C. elongatus* seem to share a similar organization of the antheridium (see Fig. 18a and d in Santamaría 2003). *Cantharomyces denigratus*, however, has a deeply pigmented receptacle. *Cantharomyces italicus* has a relatively long primary appendage but its antheridium is different, i.e. lens-shaped, small and laterally positioned within a more elongate basal cell of the primary appendage.

Cantharomyces numidicus Maire, as well as the very similar *Cantharomyces japonicus* K. Sugiy., differ in having a relatively large antheridial segment and a dissimilar arrangement of its primary appendage's basal cell (Thaxter 1931, Santamaría 2003).

Cantharomyces elongatus parasitizes *Syntomium aeneum*, extending the known host range of *Cantharomyces* to the staphylinid tribe *Euphaniini* (subfam. *Oxytelinae*).

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Lyophyllum rhombisporum sp. nov. from China

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Abstract — A new species, collected from Yunnan Province, China, is here described as *Lyophyllum rhombisporum* (*Lyophyllaceae, Agaricales*). The new species is macroscopically and microscopically very similar to *L. sykosporum* and *L. transforme*, but its spore shape is quite different. Molecular analysis also supports *L. rhombisporum* as an independent species.

Key words — *Agaricomycetes*, mushroom, taxonomy, ITS, phylogeny

Introduction

Lyophyllum is a genus in the *Lyophyllaceae* (*Agaricales*) with about 40 species, which are widespread in north temperate regions (Kirk et al. 2008). A few *Lyophyllum* species are well-known edible mushrooms, such as *L. shimeji* (Kawam.) Hongo, *L. decastes* (Fr.) Singer, and *L. fumosum* (Pers.) P.D. Orton. Research in China has reported 19 *Lyophyllum* species (Li 2002; Li et al. 2005; Dai & Tolgor 2007; Li & Li 2009), but more research is still needed on the taxonomy, molecular phylogeny, and cultivation of this genus. Here we describe a new species, *L. rhombisporum* from Yiliang County, Yunnan Province, in southwestern China.

Materials & methods

Microscopic and macroscopic characters were described based on the specimen material (L1736) following the methods of Yang & Zhang (2003). Sections were made by hand with a razor blade, then mounted in 5% KOH solution and lactophenol cotton blue respectively under an Olympus BH-2. The specimens were deposited in the Herbarium of Yunnan Academy of Agricultural Sciences, Kunming, China (YAAS).

Total DNA was extracted using the modified CTAB methodology (Hofstetter et al. 2002; Li et al. 2011). The internal transcribed spacer region (ITS) was amplified using

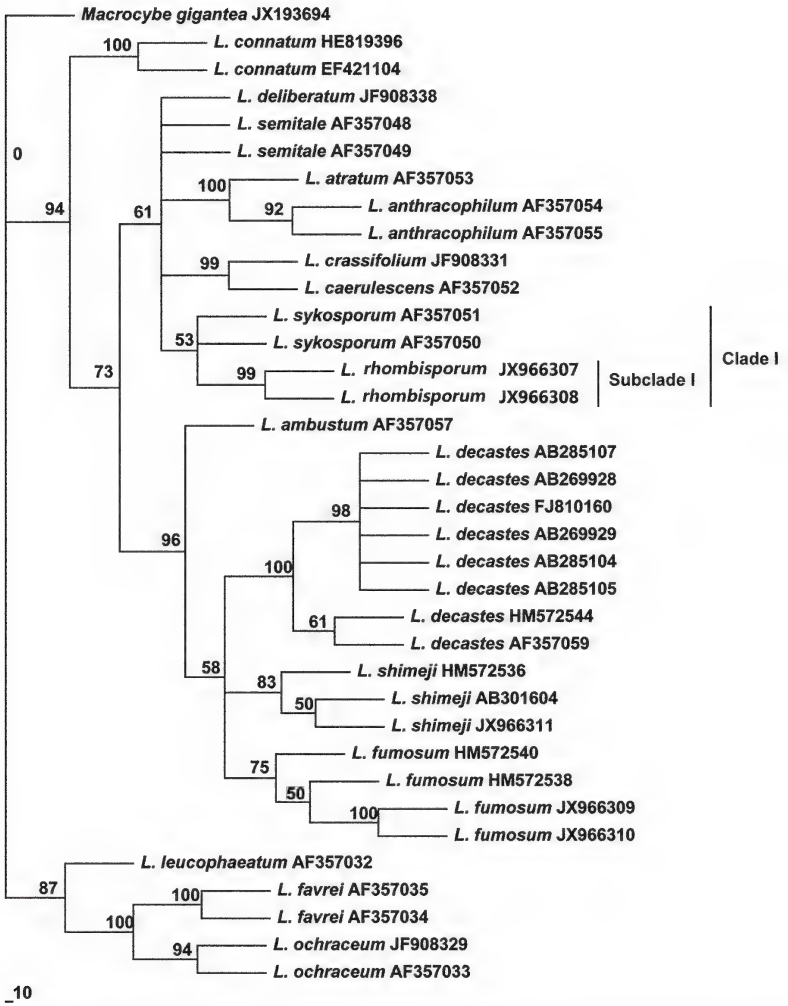

Plate 1. Equally weighted parsimony tree of the genus *Lyophyllum* based on ITS sequences from 36 ITS sequences. Numbers above branches indicate bootstrap support.

primers ITS4 and ITS5 (Hofstetter et al. 2002; Larsson & Sundberg 2011), following the modified Li's procedure (Li et al. 2011). Purifying and sequencing of PCR products were conducted by Beijing Genomics Institute.

Thirty-six sequences were included in molecular analysis: six generated from this research and another 30 retrieved from GenBank. *Macrocybe gigantea* (Massee) Pegler & Lodge was selected as the outgroup. Sequences were edited and assembled using SeqMan II (Larsson & Sundberg 2011), aligned by Muscle 3.7, and adjusted manually using BioEdit 7.0.1. Phylogenetic analyses were carried out via PAUP* 4.0 BEAT. Equally

weighted parsimony (MP) was used to search for optimal trees according to Hofstetter et al. (2002) and Larsson & Sundberg (2011).

Results & discussion

Phylogenetic analysis

Six strict consensus trees were generated based on the MP analyses of 36 ITS sequences (L = 479, CI = 0.714, RI = 0.866, RC = 0.618) (Plate 1). The phylogeny supports *L. rhombisporum* as distinct from the sampled *Lyophyllum* species, although it is closely related to *L. sykosporum*. In the strict consensus tree, *L. rhombisporum* and *L. sykosporum* form Clade I with 53% bootstrap support. This subdivides into two subclades, with Subclade I comprising *L. rhombisporum* with a strong bootstrap support (bs = 99%) and separated from Subclade II comprising *L. sykosporum*.

Taxonomy

Plate 2. Fruit-body of *Lyophyllum rhombisporum*.

Lyophyllum rhombisporum Shu H. Li & Y.C. Zhao **sp. nov.** Plates 2, 3

MycoBank MB 801752

Differs from *Lyophyllum sykosporum* and *L. transforme* by its rhombic basidiospores.

Type: China: Yunnan, Yiliang County, 24°42′N 103°09′E, alt. 1922 m, in forest of *Pinus yunnanensis* Franch, 23.6.2011, Shuhong Li, (**Holotype**, YAAS L1763; GenBank JX966307).

Etymology: Latin *rhombisporum*, referring to the rhombic or subrhombic shape of the spores.

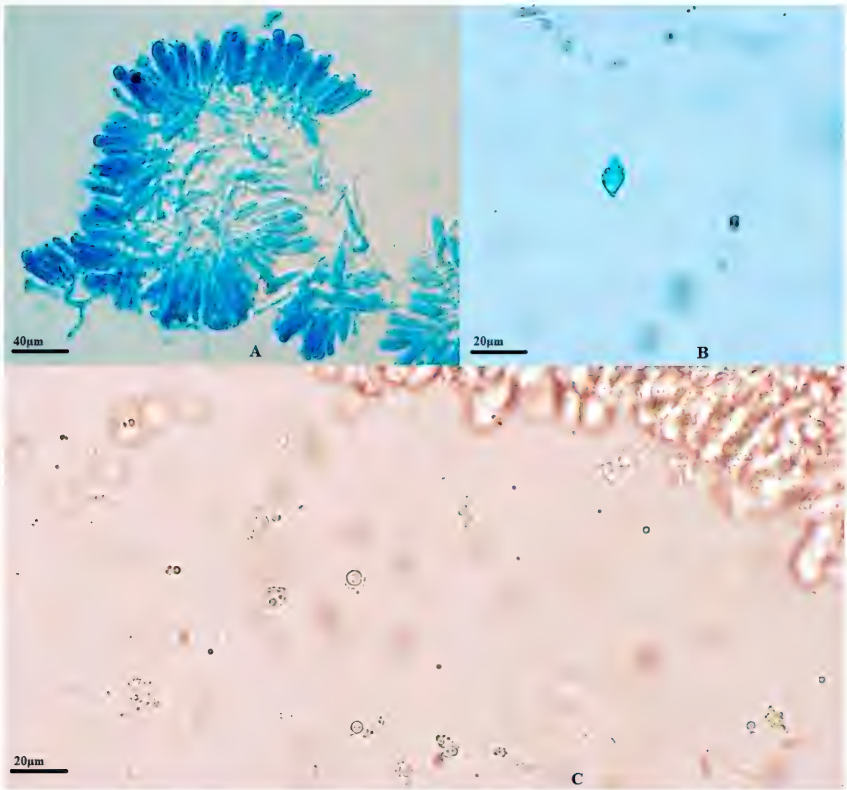

Plate 3. *Lyophyllum rhombisporum* (Holotype L1763). Fig. A. Basidia in lactophenol cotton blue; Fig. B. Spores in 5% KOH; Fig. C. Spore in lactophenol cotton blue.

Pileus 2.0–7.5 cm, convex, applanate at maturity, smooth, dry, not striate, greyish to pale taupe. Context thin, white to whitish grey. Odor and taste not distinctive. Stipe 3.0–8.0 long, 0.6–1.5 cm thick, cylindrical, greyish or greyish brown, slightly enlarged downwards, appressed fibrillose, pale brown at base. Lamellae moderately close together, adnate or subdecurrent, broad, whitish to greyish, blackening when bruised, edge blunt). Hymenophoral trama regular, consisting of hyaline hyphae, clamp-connections rare. Spores rhombic or subrhombic, hyaline, cyanophilous, with a single large oil-droplet or a few small oil-droplets, smooth, 14.5–17 × (7.5–)10–11.5 μm, Qm = 1.3. Basidia 36.5–46 × 10–11.3 μm, 4-spored, clavate, with siderophilous granulation well visible in lactophenol cotton blue or cotton blue, clamp connections absent. Pleuro- and cheilocystidia absent.

Ecology & Distribution: Scattered or gregarious in forests of *Pinus yunnanensis*. Summer to autumn. Known only from Yunnan Province, China.

Additional specimen examined: **CHINA**: **Yunnan**, Yiliang County, 24°42′N 103°09′E, alt. 1952 m, in *Pinus yunnanensis* forest, 21.9.2011, Shuhong Li, (YAAS L2082; GenBank JX966308).

Comments — This species is characterized by large sub-rhombic or rhombic basidiospores, and white or greyish gills that blacken when bruised, a feature of section *Lyophyllum* (Breitenbach & Kränzlin 1991; Hofstetter et al. 2002; Larsson & Sundberg 2011). Morphologically *Lyophyllum rhombisporum* is morphologically similar to *L. sykosporum* Hongo & Clémençon and *L. transforme* (Sacc.) Singer (Breitenbach & Kränzlin 1991; Hofstetter et al. 2002), which are distinguished by their triangular basidiospores. The phylogenetic analysis supports *L. rhombisporum* as a new species.

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The authors are grateful to Wang Yun, Zhao Ruilin, and Dr Ian R. Hall, who critically reviewed the manuscript and provided invaluable suggestions. We acknowledge Ma Tao who helped us with the microscopic technical examination. This study was financially supported by the National Science Foundation of China (No.31160010).

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New records of corticolous lichens from Vietnam

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Abstract —Eight new records of crustose lichens are reported from Dak Lak, Dak Nong, and Vinh Phuc provinces in Vietnam. Descriptions of *Diorygma hieroglyphicum*, *Letrouitia leprolytoides*, *L. transgressa*, *Ocellularia perforata*, *Opegrapha robusta*, *Platythecium colliculosum, P. dimorphodes,* and *Syncesia farinacea* include morphological, anatomical, chemical characters, and comparisons with similar taxa.

Key words—*Graphidaceae*, *Letrouitiaceae*, *Roccellaceae*, evergreen forest, tropical

Introduction

A recent survey suggested that lichen species richness increases towards lower latitudes and that tropical ecosystems harbor about half of the total lichen species known worldwide (Lücking et al. 2011). Vietnam, part of the Eastern palaeotropical region, provides a climate suitable for lichens, particularly crustose, corticolous, and foliicolous species (Nguyen et al. 2010, 2011). Sporadic surveys and lichen collections from different regions of this country indicate that the majority of its land is still unexplored for its lichen diversity. Aptroot & Sparrius (2006) provided the first checklist of Vietnamese lichens, but no update has been published since. However, recent publications (Giao 2009; Nguyen et al. 2010, 2011) on foliose and foliicolous lichens in Vietnam document the potential richness in lichens in this region, and suggest that many more species have not yet been recorded.

We report here eight corticolous species representing *Diorygma*, *Letrouitia*, *Ocellularia*, *Opegrapha*, *Platythecium*, and *Syncesia* as new for Vietnam. Species in four of these genera have previously been recorded from Vietnam: *Diorygma tinctorium* Eschw., *Letrouitia domingensis* (Pers.) Hafellner & Bellem., *Ocellularia allosporoides* (Nyl.) Patw. & C.R. Kulk., *O. asiatica* (Vain.) Hale,

O. dolichotata (Nyl.) Zahlbr., *O. papillata* (Leight.) Zahlbr., *O. tenuis* (Hale) Hale, *O. thelotremoides* (Leight.) Zahlbr, *Opegrapha filicina* (Mont.) Müll. Arg., *Op. fuscovirens* Müll. Arg., *Op. varia* var. *heterocarpa* Müll. Arg., and *Op. vegae* R. Sant. No species of *Platythecium* and *Syncesia* were recorded in the checklist of Vietnamese lichens (Aptroot & Sparrius 2006).

Materials and methods

Lichen specimens were collected during April 2012 from Chu Yang Sin and Yok Don National Parks in Dak Lak province, and Dray Nur Waterfall in Dak Nong province in the central highlands and Tam Dao National Park in Vinh Phuc province in northern Vietnam. The specimens were conserved in the lichen herbarium of the Korean Lichen Research Institute, Suncheon, South Korea (KoLRI).

The specimens were examined using standard light microscopy techniques. Sections of thalli and ascocarps were mounted in water, 10% KOH, lactophenol blue, and Lugol's solution. All measurements were made in water. Chemical constituents were identified by spot color reaction tests and thin layer chromatography (TLC; solvent system C) following published protocols (Orange et al. 2010). Measurements of hymenium, exciple, asci, and ascospores were made on 10–15 thin, hand-cut apothecial sections. The following literature was used for species identification: Aptroot & Sparrius 2006; Kalb et al. 2004; Tehler 1997; Staiger 2002; Frisch et al. 2006; Archer 2009; Elix 2009; Ertz 2009; Mangold et al. 2009; Rivas Plata et al. 2010; Joshi et al. 2011; Nguyen et al. 2010, 2011.

Taxonomy

Diorygma hieroglyphicum (Pers.) Staiger & Kalb, Symb. Bot. Upsal. 34(1): 1151, 2004. Pl. 1a

Thallus corticolous, epiphloeodal, ecorticate, ≤200 µm thick, ± verrucose due to emerging ascocarps, marginally smooth, dull, whitish to pale green; prothallus distinct, whitish; algal layer well developed, continuous; medulla distinct, white. Vegetative propagules absent. Ascocarps numerous, lirellate, immersed, straight to bend, branched, stellate, ≤2 × 0.25 mm; disc exposed, flat to concave, pale brown to brown, densely white pruinose. Thalline margin prominent, 90–100 µm wide. Proper exciple hyaline to pale brown or brown, divergent, poorly developed, 15–20 µm thick. Epihymenium pale to brownish, granular, crystalline, 10–20 µm high. Hymenium hyaline, not inspersed (but disintegrated ascospore locules sometimes have the appearance of large oil droplets), 100–135 µm high. Subhymenium hyaline to pale at the base, 30–35(–50) µm high. Paraphyses simple with branched anastomosing apices, 1–2 µm thick. Asci clavate, 1-spored, 90–130 µm, I–. Ascospores hyaline, muriform, ellipsoidal, with round ends, 65–85 × 17–29 µm, I+ blue.

Chemistry—Thallus K+ yellow orange, Pd+ reddish, C–, KC–. Stictic acid detected with TLC.

Distribution & ecology—This corticolous species is widely distributed in the Eastern palaeotropical region. It is known from Australia, Cameroon, Tanzania, Indonesia, Singapore, Papua New Guinea, Philippines, New Caledonia, Vanuatu, Solomon Islands, and Norfolk Island (Archer 2009; Kalb et al. 2004). In Vietnam, the species was collected on trunks of trees in an evergreen forest at an altitude of about 1000 m.

Specimen examined – **VIETNAM. Vinh Phuc province:** Tam Dao National Park, 21°27′10.8″N 105°38′58.2″E, alt. 987 m, on tree trunk, 25 April 2012, Hur, Oh & Nguyen VN120351 (KoLRI).

Remarks—*Diorygma hieroglyphicum* resembles *D. pruinosum* (Eschw.) Kalb et al. in having open, pruinose apothecial discs, 1-spored asci, and muriform ascospores, but *D. pruinosum* contains protocetraric acid in the thallus whereas *D. hieroglyphicum* contains stictic acid.

Letrouitia leprolytoides S.Y. Kondr. & Elix, Australas. Lichenol. 62: 17, 2008. Pl. 1B

Thallus corticolous, epiphloeodal, corticate, thin (ca. 150 µm thick) inconspicuous to well developed, pale greenish, greenish yellow to pale yellow or orange; algal layer well developed, continuous; medulla indistinct to endophloeodal, white, crystalline. Isidia coralloid, spread throughout the thallus. Ascocarps numerous, apothecioid, biatorine (but appearing lecanorine), ± rounded to more often irregular, solitary to marginally fused, emergent, sessile, orange, ≤2.5 mm diam.; disc exposed, ± flat, epruinose, reddish to dark brown, entire, ca. 2 mm diam.; margin prominent, smooth, shiny, brighter than the disc, orange, entire, ≤0.3 mm. Thalline margin reduced to absent. Proper exciple hyaline to pigmented, made up of radiating hyphae, ≤130 µm thick. Epihymenium orange brown to brownish, pigmented, 10–15 µm high. Hymenium hyaline, not inspersed, ≤70–100 µm high. Paraphyses slightly branched, ± interwoven or anastomosing, 1–1.5 µm thick. Hypothecium hyaline to slightly reddish in lower side, ≤70 µm high. Asci clavate, 8-spored, 70–95(–100) ×13–16 µm, outer wall I+ blue. Ascospores hyaline, transversely septate, fusiform to oblong or ellipsoidal, with narrowly rounded to subacute ends, non-halonate, 20–27 × 6–12 µm, with 5–9 locules; locules ± rounded to slightly angular, ± lentiform, septa moderately thick, I– .

Chemistry—Thallus K+ purple, Pd–, C–, KC–; apothecia K+ purple violet. Anthraquinones detected with TLC (greenish gray spot at Rf 5 and yellow at 7).

Distribution & ecology—*Letrouitia leprolytoides* is common in monsoon forests in Asia and has a pantropical distribution (Kondratyuk & Elix 2008; Elix 2009). In Vietnam, it was reported from the tree trunks in evergreen forests at 300–800 m altitude.

Specimens examined – **VIETNAM. Dak Nong province:** Dray Nur Waterfall, 12°33′06.3″N 107°53′19.6″E, alt. 371 m, on tree trunk, 19 April 2012, Hur, Oh & Nguyen

VN120006 (KoLRI). **Dak Lak province:** Yok Don National Park, 12°51′20.0″N 107°45′58.1″E, alt. 760 m, on tree trunk, 22 April 2012, Hur, Oh & Nguyen VN120205, VN120212, VN120218 (KoLRI).

Remarks—This species can be distinguished from most other *Letrouitia* species by its isidiate thallus. It resembles *L. leprolyta* (Nyl.) Hafellner and *L. corallina* (Müll. Arg.) Hafellner, which also have an isidiate thallus, but *L. leprolyta* has erumpent to flat, wart-like isidia and *L. corallina* has flattened and unbranched isidia and 1–2-spored asci.

Letrouitia transgressa (Malme) Hafellner & Bellem.,
Nova Hedwigia 35: 710, 1983. Pl. 1C

Thallus corticolous, epiphloeodal, corticate, thin, 55–80 µm, smooth to verrucose, pale green to olive green or olivaceous; prothallus distinct, whitish; algal layer well developed, continuous; medulla indistinct to white, crystalline. Vegetative propagules not seen. Ascocarps numerous, apothecioid, biatorine (but appearing lecanorine), round to irregular, solitary to marginally fused, emergent, sessile, ≤1.9 mm in diam.; disc exposed, flat to convex, epruinose, reddish brown to brown, ≤1.7 mm in diam; margin prominent, smooth, shiny, paler than the disc, pigmented, ca. 0.2 mm thick. Thalline margin absent. Proper exciple hyaline to pigmented, made up of radiating hyphae, 90–150 µm thick. Epihymenium orange brown, pigmented, 10–25 µm high. Hymenium hyaline, not inspersed, 70–125 µm high. Hypothecium reddish brown, ≤105 µm high. Paraphyses thin, septate, rarely branched and anastomosed, conglutinate, 1–1.5 µm thick. Asci clavate, 2–4(–8)-spored, 100–135 × 20–27 µm, I–. Ascospores hyaline to pale when mature, primarily 6–9 transversely septate, becoming progressively submuriform (1–3 vertical septa), ellipsoidal, with round to subacute ends, non halonate, 35–40(–50) × 13–17(–20) µm, I–.

Chemistry—Thallus K–, Pd–, C–, KC–; apothecia K+ purple. Anthraquinones detected with TLC (greenish gray spot at Rf 5 and yellow at 7).

Distribution & ecology— This corticolous species is common in Africa, Asia, Australia, New Caledonia, Papua New Guinea, and South America (Hafellner 1983; Elix 2009). In Vietnam, it is widespread on trees in evergreen forests at an altitude 600–1100 m.

Specimens Examined – **VIETNAM. Dak Lak Province:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′34.9″E, alt. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen VN120272 (KoLRI); 12°28′04.4″N 108°20′39.0″E, alt. 622 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen VN120286 (KoLRI); 12°28′12.3″N 108°20′59.9″E, alt. 763 m, on tree branch, 20 April 2012, Hur, Oh & Nguyen VN120242 (KoLRI). **Vinh Phuc province:** Tam Dao National Park, 21°27′21.7″N 105°39′00.8″E, alt. 1092 m, on tree trunk, 25 April 2012, Hur, Oh & Nguyen VN120333 (KoLRI).

Remarks—*Letrouitia transgressa* is similar to *L. parabola* (Nyl.) R. Sant. & Hafellner in having submuriform ascospores at maturity but differs in having

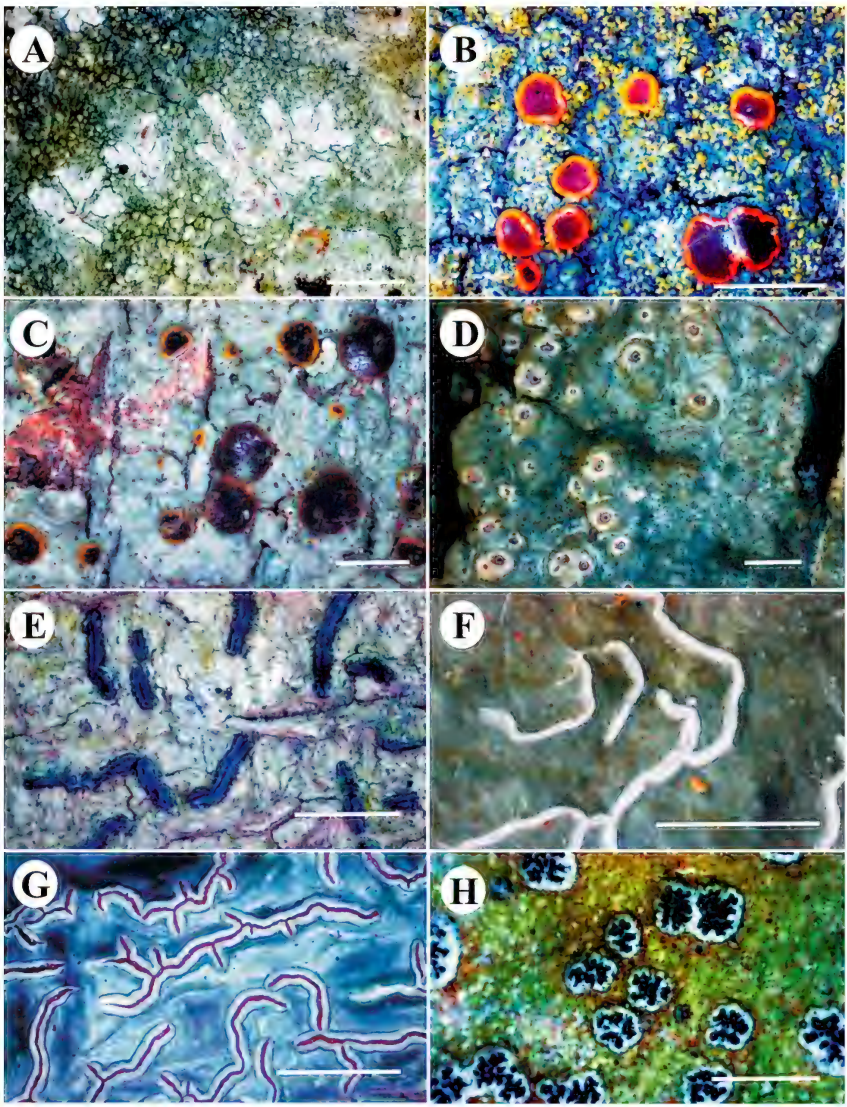

Plate. 1. New records. A. *Diorygma hieroglyphicum*. B. *Letrouitia leprolytoides*. C. *Letrouitia transgressa*. D. *Ocellularia perforata*. E. *Opegrapha robusta*. F. *Platythecium colliculosum*. G. *Platythecium dimorphodes*. H. *Syncesia farinacea*. Scale bars: A, B, G = 3 mm; C = 1.5 mm; D, E = 1 mm; F = 10 mm; H = 5 mm.

ascospores with transverse septation and lens-shaped lumina in the primary stage. In contrast, the ascospores of *L. parabola* have a spiral septation. Moreover, *L. parabola* has slightly smaller ascospores with fewer septa (6–8 locules).

Letrouitia Hafellner & Bellem. comprises ca. 18 species world-wide. Most *Letrouitia* species have similar morphological and chemical features and have been segregated according to ascospore size and septation (Hafellner 1983; Elix 2009). Generally three septation types are found in this genus: transverse, spiral, and muriform. More frequently, ascospores with either transverse or spiral septation become (sub-)muriform at maturity. As the taxonomic value of ascospore septation in lichenized fungi has been questioned (Salisbury 1978; Galloway & Guzmán 1988), molecular data is needed for the revision of *Letrouitia*.

Ocellularia perforata (Leight.) Müll. Arg., Hedwigia 31: 284, 1892. Pl. 1D

Thallus corticolous, epiphloeodal, corticate, ≤400 µm thick, continuous to ± verrucose, smooth to rough, dull to ± glossy, greenish gray or pale to olivaceous green; algal layer well developed, continuous; medulla thin to indistinct, white, with small to moderately large, clustered or scattered calcium oxalate crystals. Vegetative propagules absent. Ascocarps numerous and conspicuous, porinoid, becoming apothecioid, hemispherical, ± rounded to irregular at maturity, solitary to fused, immersed to ± raised, rarely strongly emergent, ≤1 mm diam.; disc with the columella visible at maturity, epruinose to slightly pruinose, flesh-colored to pale or brownish; pore formed by the rim of the thalline margin, round to irregular at maturity, entire, to 0.5 mm diam. Thalline margin has the same color as the thallus or slightly paler, thin to thick, ± incurved. Proper exciple fused, brownish to dark brown, ca. 120 µm thick. Epihymenium hyaline, with grayish or brownish granules, ≤15 µm high. Hymenium hyaline, not inspersed, ≤150 µm high. Paraphyses straight to bent, unbranched, with slightly thickened tips, ± interwoven, moderately to distinctly conglutinated, 1–1.5 µm thick. Columellar structure well developed at maturity, entire, brownish to carbonized, ± covered by grayish or brownish granules, to 300 µm thick. Subhymenium indistinct to hyaline. Asci clavate, 8-spored, (75–)100–120 µm, I–. Ascospores hyaline, transversely septate, oblong to ellipsoidal, with ± rounded to narrowly rounded or subacute ends, thick walled, non halonate, 20–32 × 6–9 µm, with 8–10 locules, I+ blue violet; locules ± rounded to slightly angular, oblong to lentiform, with hemispherical to conical end cells.

Chemistry—Thallus K+ yellowish brown, Pd+ orange red, C–, KC–. Protocetraric acid detected with TLC.

Distribution & ecology— *Ocellularia perforata* has a pantropical and southern-temperate distribution (Mangold et al. 2009) and has been reported from rainforests. In Vietnam, it was collected from tree trunks in evergreen forests at an altitude of ca.1000 m.

Specimen examined – **VIETNAM. Vinh Phuc province:** Tam Dao National Park, 21°27′21.7″N 105°39′00.8″E, alt. 1092 m, on tree, 25 April 2012, Hur, Oh & Nguyen VN120323 (KoLRI).

Remarks—*Ocellularia perforata,* which is highly morphologically variable, is a widespread species. The morphologically similar *O. papillata* and *O. terebrata* (Ach.) Müll. Arg., can be distinguished by their thallus chemistry: the thallus of *O. terebrata* produces psoromic acid, whereas *O. papillata* lacks secondary metabolites.

Opegrapha robusta Vain., Bot. Tidsskr. 29: 137, 1909. Pl. 1E

Thallus corticolous, endo- to epiphloeodal, ecorticate (cortex not clearly distinguishable in section), thin (≤150–200 µm thick), ± inconspicuous, spread in large patches, dull to slightly glossy, pale-green to green, olive green or dark brownish; prothallus distinctly dark brown to black; algal layer well developed, continuous; medulla indistinct, white. Vegetative propagules absent. Ascocarps lirellate, numerous and evenly distributed on the thallus; lirellae simple to slightly furcate, sessile, black, epruinose, straight to curved, ± flexuose, reaching ≤7 × 0.25 mm. Thalline exciple basal to absent. Proper exciple completely carbonized, convergent, K+ olivaceous, entire, 130–160 µm thick. Epihymenium indistinct to pale. Hymenium hyaline, not inspersed, gelatinous, ca. 150 µm high. Hypothecium hyaline to yellowish, ≤25 µm high. Paraphyses richly anastomosing, 1–1.5 µm thick. Asci ellipsoid to clavate, 8-spored, 80–115 × 20–24 µm, K/I+ blue apical ring distinct. Ascospores hyaline, fusiform, transversely 9–15-septate, 40–70 ×5–9 µm (including a perispore ≤2 µm thick), not constricted at septa, I–.

Chemistry—Thallus K–, Pd–, C–, KC–. Confluentic acid detected with TLC.

Distribution & ecology—*Opegrapha robusta* is known from Africa (Gabon, Zaire), Asia (Indonesia, Malaysia, Papua New Guinea, The Philippines, Thailand), Australia, and Solomon Islands (Ertz 2009). In Vietnam, it was recorded on trees in dense, tropical evergreen forests at an altitude of 600–800 m.

Specimens examined – **VIETNAM. DakLak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39.0″E, alt. 622 m, on tree trunk, 20 April 2012, Hur, Oh & Nughen VN120290 (KoLRI); 12°27′57.0″N 108°20′34.9″E, alt. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nughen VN120275 (KoLRI).

Remarks—*Opegrapha robusta* is well separated from the two other similar species *Op. prosodea* Ach. and *Op. viridis* (Ach.) Behlen & Desberger in having robust lirellate apothecia and confluentic acid. *Opegrapha prosodea* and *Op. viridis* have smaller apothecia (1–3 mm long) and lack secondary compounds.

Platythecium colliculosum (Mont.) Staiger, Biblioth. Lichenol. 85: 380, 2002. Pl. 1F

Thallus corticolous, epiphloeodal, corticate, ≤120 µm thick, continuous, smooth or irregularly to scarcely verrucose (due to the substrate texture),

± glossy, olive to grayish green; prothallus distinct, white; algal layer well developed, continuous; medulla distinct, white. Vegetative propagules absent. Ascocarps lirellate, numerous and scattered on the thallus; lirellae emergent, straight to bend, radially to irregularly branched, with acute ends, 10–20 × 0.25 mm (but the frequent branching increases the length of lirellae ≤150 mm); labia closed to open, white, crenate in mature apothecia, ≤133 µm thick; disc reddish brown to brown (not well exposed in the examined specimen). Thalline margin complete, 30–35 µm thick. Proper exciple indistinct to brownish, ± striate. Epihymenium brownish, crystalline to granular, 9–10 µm high, I+ blue. Hymenium hyaline, not inspersed, 80–90 µm high, I+ slightly blue. Subhymenium well developed, hyaline to pale yellow, ≤45 µm high, I–. Paraphyses simple at the base, but apically irregularly branched, 1–2 µm thick. Asci clavate, 8-spored, 60–70 × 12–15 µm, I–. Ascospores hyaline, submuriform, ellipsoidal to ovoid, transversely 4-locular with one vertical septum in middle locules, 9–13 × 6–8 µm, I+ blue violet.

Chemistry—Thallus K+ red, Pd+ yellow orange, C–, KC–. Salazinic acid detected with TLC.

Distribution & ecology—*Platythecium colliculosum* occurs most commonly on bark. It is distributed mostly in tropical and subtropical regions of the world. In Vietnam, the species was reported from trees in an evergreen forest at an altitude between700–800 m.

Specimen examined – **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′34.9″E, alt. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen VN120135 (KoLRI).

Remarks—The Vietnamese specimen of *P. colliculosum* shows a slight chemical variation. Staiger (2002) described the species as producing both salazinic and protocetraric acids, but we detected only salazinic acid in our material. The morphologically similar species *P. dimorphodes* differs in containing norstictic acid in the thallus.

Platythecium dimorphodes (Nyl.) Staiger, Biblioth. Lichenol. 85: 383, 2002. Pl. 1G

Thallus corticolous, epiphloeodal, corticate, 200–500 µm thick, smooth to uneven, irregularly to scarcely verrucose, shiny to ± matt, grayish-green to greenish; algal layer well developed; medulla distinct, white. Vegetative propagules absent. Ascocarps lirellate, numerous, emergent, straight to bend, irregularly branched, 10 × 0.25–0.7 mm; labia open, white, crenate in mature apothecia; disc reddish brown to brown, exposed, flat to slightly concave, occasionally with a thin whitish pruina. Thalline margin complete, ± undulating, 50–75 µm thick. Proper exciple pale brown to brown, simple to ± divided laterally in mature apothecia. Epihymenium brownish, crystalline to granular, 12–14 µm high, I–. Hymenium hyaline, not inspersed, 80–90 µm

high, I–. Subhymenium well developed, hyaline to yellowish, 40–45 µm high, I–. Paraphyses± anastomosed, apically irregularly branched, 1–1.5 µm thick. Asci clavate, 8-spored, 70–80 × 13–15 µm, I–. Ascospores hyaline, ellipsoidal to ovoid, submuriform, with three transverse septa and one vertical septum in the middle locule, 15–17 × 7–8 µm, I+ blue violet.

Chemistry—Thallus K+ yellowish, Pd+ yellow orange, C–, KC–. Norstictic acid detected with TLC.

Distribution & ecology—The species is common in the Eastern palaeotropical countries and is distributed in tropical and subtropical regions of Australia, Malaysia, Java, Papua New Guinea, Philippines, and Sri Lanka (Staiger 2002; Archer 2009). In Vietnam, it was reported from trees in an evergreen forest at an altitude of 600–800 m.

Specimens examined – **VIETNAM. Dak Lak Province:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′32.9″E, alt. 780 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen VN120123 (KoLRI); 12°27′57.0″N 108°20′34.9″E, alt. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen VN120176 (KoLRI); 12°28′04.4″N 108°20′32.0″E, alt. 622 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen VN120284 (KoLRI).

Remarks—*Platythecium colliculosum,* which superficially resembles *P. dimorphodes*, differs in containing salazinic and protocetraric acids as thallus compounds. *Platythecium dimorphodes,* which has immature entire labia and apothecial disc color similar to those in *P. grammitis* (Fée) Staiger and *P. leiogramma* (Nyl.) Staiger, differs in its lack of secondary metabolites. The Vietnamese collections differ from *P. dimorphodes* as originally described (Staiger 2002) in lacking isidia-like structures.

Syncesia farinacea (Fée) Tehler, Flora Neotropica, Monogr. 74: 25, 1997. Pl. 1h

Thallus corticolous, epiphloeodal, ecorticate, 100–200 µm thick, tomentose to dense, coherent, homoiomerous, water-absorbent, ± rimose, smooth to slightly verrucose, slightly pruinose, creamy-white to ± greenish, UV+ cream to yellowish; prothallus dark brown; calcium oxalate crystals absent; surface gel absent; medulla usually indistinct; hypomedullary plectenchyma absent. Ascomata numerous, pseudo-monocarpocentral, sessile with constricted base, ≤3 mm diam.; discs flat to convex, ≤0.5 mm diam., tomentum sometimes present, but usually absent. Thalline margin at the same level than the disc, ecorticate, but with algae. Proper exciple ± inconspicuous to ca. 50 µm thick. Epihymenium dark brown, hyphae richly branched, not separable, verrucose, brownish, 13–20 µm high, I+ intense blue, KI+ blue. Hymenium hyaline, clear, 70–100 µm high, I+ blue. Paraphyses rather lax, 2–3 µm thick, branched, I–, KI+ blue. Hypothecium dark brown, ± carbonaceous, extending down to the substrate, 200–400 µm high, K+ olive green, I–, KI–. Asci, clavate, 8-spored, 60–70 × 10–15 µm, I+ blue. Ascospores hyaline, transversely 3-septate, fusiform, ± curved, 25–30 × 3–5 µm, I–.

Chemistry—Thallus K+ reddish, Pd–, C–, KC–. Traces of protocetraric and roccellic acids detected with TLC.

Distribution & ecology—This variable species occurs mostly on bark of trees and shrubs but also sometimes on rocks. Reported from both the northern and southern hemispheres, *S. farinacea* is known from Mexico, the West Indies, Colombia, Venezuela, and Brazil (Tehler 1997). In Vietnam, the taxon was found growing luxuriantly over tree trunks at an altitude between 600–800 m in an evergreen forest.

Specimens examined – **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′34.9″E, alt. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen VN120175, VN120177 (KoLRI); 12°28′04.4″N 108°20′39.0″E, alt. 622 m, on tree trunk,21 April 2012, Hur, Oh & Nguyen VN120285 (KoLRI).

Remarks—We identify our specimens as *Syncesia farinacea,* despite thallus color and texture differences from the type description that may be due to different environmental conditions. *Syncesia graphica* (Fr.) Tehler resembles *S. farinacea* but differs in having lecanoric acid (C+ red thallus) and erythrin as secondary compounds; moreover, in *S. farinacea* the synascomata are more rounded and constricted at the bases and the ascospores are somewhat smaller. Another similar species, *S. effusa* (Fée) Tehler, can be distinguished by its UV+ bright light yellow thallus and the production of atranorin as an additional compound. *Syncesia farinacea* can sometimes be confused with *S. rhizomorpha* Tehler, which also has rhizomorph-like structures but which is distinguished by smaller ascospores, calcium oxalate crystals in the thalline margin, and schizopeltic acid in the thallus.

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MYCOTAXON

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Volume 123, pp. 491–492 *January–March 2013*

Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce five new species distribution lists to our 'web-list' page covering gasteroid fungi (by Hernández-Caffot & al.) and *Paxillaceae, Gomphidiaceae*, *Boletaceae* and *Russulaceae* (by Niveiro & Albertó) in Argentina; lichens in Armenia (by Gasparyan & Sipman); corticioids in the Canary Islands, Spain (by Beltrán-Tejera & al.); and the lichens of Iğdır province, Turkey (by Yazici & al.). This brings to 105 the free access mycobiotas now available on the Mycotaxon website:

<http://www.mycotaxon.com/resources/weblists.html>.

South America

Argentina

María L. Hernández-Caffot, Gerardo Robledo & Laura S. Domínguez. Gasteroid mycobiota (*Basidiomycota*) from *Polylepis australis* woodlands of central Argentina. (12 p.)

Abstract — Gasteroid fungi associated with *Polylepis australis* forests from Córdoba Mountain range, central Argentina, were studied. Samples were collected during April-May 2007. Twenty-nine species were identified among which *Bovista pila*, *Geastrum morganii* and *Tulostoma xerophilum* are new records for Argentina and the distribution range of *Bovista nigrescens*, *Cyathus olla*, *Geastrum fornicatum* and *Lycoperdon pyriforme* are expanded to high altitude woodlands from Córdoba.

N. Niveiro & E. Albertó. Checklist of the Argentinean *Agaricales* 6. *Paxillaceae*, *Gomphidiaceae*, *Boletaceae* and *Russulaceae*. (12 p.)

Abstract — A checklist of species belonging to families *Paxillaceae*, *Gomphidiaceae*, *Boletaceae,* and *Russulaceae* was made for Argentina. The list includes all species published up to 2011. Sixteen genera and 54 species are recorded: 15 species from *Paxillaceae*, 3 from *Gomphidiaceae*, 11 from *Boletaceae,* and 25 from *Russulaceae*.

Europe

Armenia

Arsen Gasparyan & Harrie J. M. Sipman. New lichen records from Armenia. (5 p.)

Abstract — Nineteen species are added to the known lichen mycota of Armenia. Three of these, *Lecanora wetmorei, Lecanora percrenata* and *Lecanora flowersiana,* are of particular interest because they are currently predominantly known from North America, and one, *Gyalecta truncigena*, because it represents a genus new for Armenia.

Spain

Esperanza Beltrán-Tejera, J. Laura Rodríguez-Armas, M. Teresa Telleria, Margarita Dueñas, Ireneia Melo, M. Jonathan Díaz-Armas, Isabel Salcedo & José Cardoso. Corticioid fungi from arid and semiarid zones of the Canary Islands (Spain). Additional data. 2. (27 p.)

Abstract — A study of the corticioid fungi collected in the arid, semiarid, and dry zones of the Canary Islands is presented. A total of eighty species, most of them growing on woody plants, was found. Nineteen species are reported for the first time from the archipelago (*Asterostroma gaillardii, Athelia arachnoidea, Botryobasidium laeve, Byssomerulius hirtellus, Candelabrochaete septocystidia, Corticium meridioroseum, Crustoderma longicystidiatum, Hjortstamia amethystea, Hyphoderma malençonii, Leptosporomyces mutabilis, Lyomyces erastii, Peniophora tamaricicola, Phanerochaete omnivora, Phlebia albida, Radulomyces rickii, Steccherinum robustius, Trechispora praefocata, Tubulicrinis incrassatus,* and *T. medius*). The importance of endemic plants, such as *Rumex lunaria, Euphorbia lamarckii, E. canariensis, Kleinia neriifolia, Echium aculeatum*, and *Juniperus turbinata* ssp. *canariensis*, inter alia, as substrates for corticioid fungi was analyzed. The distribution of these fungi in different types of xerophytic plant communities and bioclimatic belts are discussed, as well as their ecological implications.

Mid-east

Turkey

Kenan Yazici, Andre Aptroot & Ali Aslan. The lichen biota of Iğdır province (Turkey). (26 p.)

Abstract — As a result of lichenological exploration in the Iğdır province (Turkey), a total of 292 lichenized fungi belonging to 96 genera in the *Ascomycota*, including 2 subspecies and 6 varieties, were determined from 174 different localities. *Caloplaca glomerata*, *Caloplaca kansuensis*, *Dermatocarpon miniatum* var. *cirsodes*, *Lecanora bicincta* var. *sorediata*, *Lecanora prophetae-eliae*, *Phaeophyscia hispidula*, *Physcia scopulorum*, *Placopyrenium iranicum* and *Pyrenopsis subareolata* are new to Turkey, and also Asia except *Pyrenopsis subareolata* and *Lecanora prophetae-eliae*.

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